STUDIES ON SILK PRODUCTION AND SPINNING BEHAVIOUR IN SPIDERS AND SILKWORMS

by

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CONTENTS

INTRODUCTION	page	1.
SILKWORMS AND SPIDERS AS MEDIA FOR BEHAVIOURAL STUDIES		5.
EXPERIMENTAL APPROACHES IN PHYSIOLOGICAL STUDIES OF BEHAVIOUR		8.
SILK SPINNING IN SPIDERS		16.
A. The spinning apparatus		17.
1. Structure of the glands, spigots and spinnerets		17.
2. Production of silk, and movements of the spinnerets during spinning		20.
3. Control valve operation		22.
B. The mechanics of silk spinning		25.
1. Experimental technique		26.
2. Results		27.
SILK SPINNING IN SILKWORMS		29.
RESPIRATORY METABOLISM AND SPINNING BEHAVIOUR IN SILKWORMS		32.
A. The relation of respiratory rate to spinning periods		32.
1. Materials and methods		32.
2. Changes in respiration during normal larval moulting		33.
3. Changes in respiration during the onset of normal metamorphosis		34.
B. The effect of starvation on the respiration and behaviour of feeding silkworms		35.
C. The relation of glandectomy to respiratory rate and spinning behaviour		36.
1. Material and methods		38.
2. Effects of removing silk glands on respiratory rate		40.
3. Effects of removing silk glands on spinning movements		41.
D. The suppression of respiratory metabolism and its effect on spinning behaviour		42.
1. Use of phenylthiourea to depress respiratory metabolism		43.
2. Effect of phenylthiourea on blood phenolase in feeding stages		45.

3. Effect of suppressing respiratory rate upon cocoon construction 46.

E. Reducing metabolism by exposure to low temperatures and its effect on larval movement 50.

F. The brain in relation to respiratory systems 54.

1. Tracheation of the brain 54.

2. Effects of severing the tracheal supply to the brain on behaviour 54.

DISCUSSION 56.

1. Silk spinning 56.

2. Depressant action of phenylthiourea on respiration 58.

3. Control of spinning behaviour 60.

4. Relation of metabolic rate to behaviour 69.

SUMMARY 73.

LITERATURE CITED 75.

ACKNOWLEDGEMENTS 80.

PUBLICATION OF RESULTS 81.

INTRODUCTION

The cocoon of the silkworm (Figure 1) and the web of the spider (Figure 2) are both end results of a complicated series of neuromuscular activities performed during spinning. They are tangible structures, the design of which can be predicted, and they reflect the associated spinning behaviour pattern. Experimentally induced changes in the behaviour pattern therefore tend to be mirrored in these structures as observable alterations in their design, which is sufficiently complex to reveal slight abnormalities. These abnormalities can therefore be used to estimate the changes in the behaviour pattern consequent on some experimental treatment, and the practice of examining the cocoon or web to detect such abnormalities therefore conveniently obviates the necessity for complete reliance on subjective impressions. The time scale of behavioural experiments is usually so long as to render continuous observation difficult, if not impossible, and it is therefore convenient to choose for study an animal which spins its own record. Each strand in the cocoon represents a single movement of the head of the silkworm, while each thread in the web of the spider represents a single pattern of movement, involving the spinnerets and the legs.

It therefore seemed that the study of silk production and spinning behaviour in both spiders and silkworms might prove rewarding in making a physiological approach to the problem of how behaviour patterns are initiated, modulated and controlled.

It is well known that changes in overall metabolism seem to accompany fundamental changes in behaviour. For example, the solitary and migratory phases of locusts have been linked with

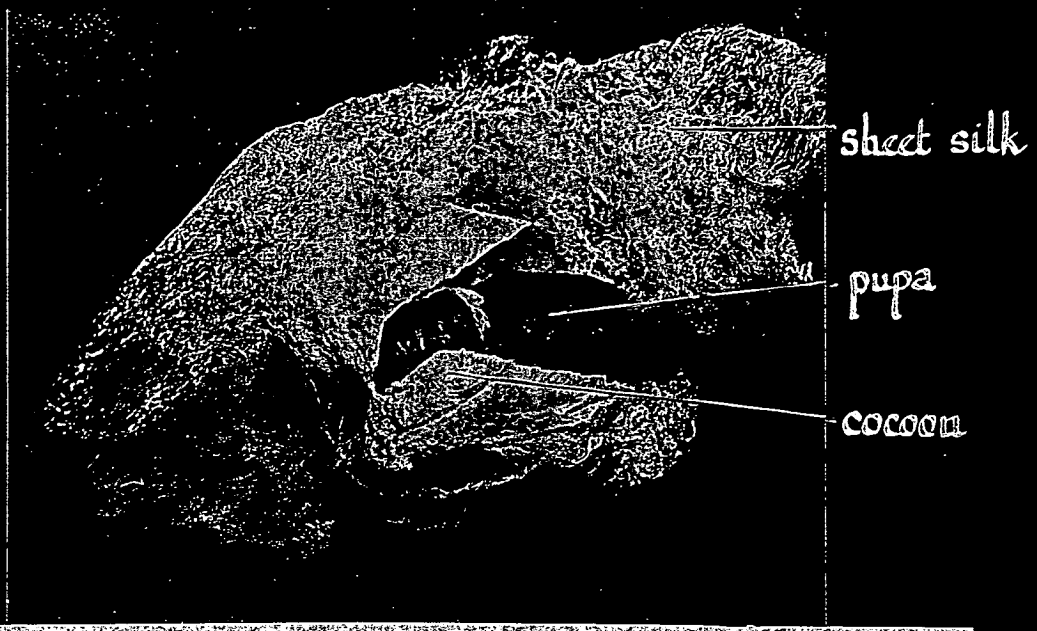

Figure 1. Cocoon spun by normal Cynthia larva, cut open to show the pupa inside. The cocoon was spun inside a glass vial, and was attached to the glass by the sheet silk. (x1½ approx.)

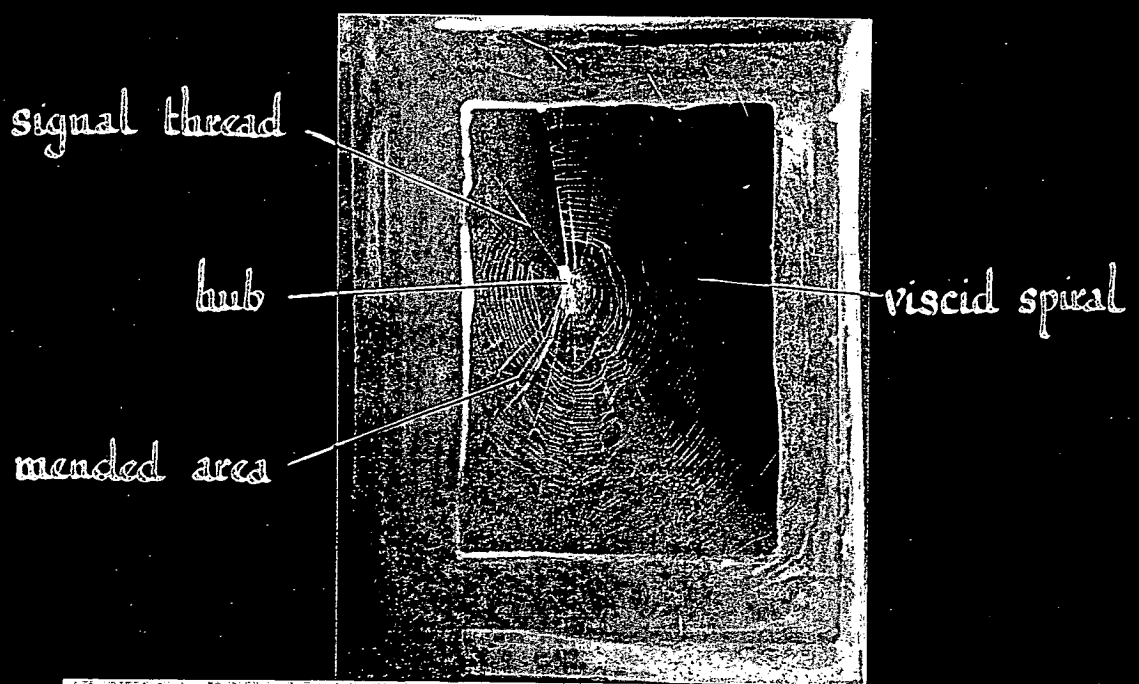

Figure 2. Web spun by adult female Zygiella inside cardboard box. The partially mended breaks in the web indicate where flies have been caught. (x1/3 approx.)

different states of metabolism, that of the migratory phase being markedly higher. The extensive literature on the honey-bee contains many indications that the sequence of duties performed by the workers, and indeed that the whole regulation of the colony, is closely bound up with metabolism. Worker bees will perform duties according to the needs of the colony, rather than according to their own "normal" sequence, which fact clearly indicates the closeness of the link between their metabolic state and overt behaviour. As a vertebrate example, one may mention bird migration as another behavioural phenomenon which is known to be correlated in some way with a basic metabolic change.

Up till now, however, it would seem that the influence of metabolism on behaviour has been regarded as a general effect, and this may well be true in those cases just mentioned. Yet at the present time, when efforts are being made to gain further insight into the causes of abnormal human behaviour, significant links between metabolic rates and modes of behaviour have been observed in some types of mental illness. The problem of relating metabolic rates to subtle changes in behaviour at the mammalian or avian levels, is, of necessity, exceedingly complex, and it might therefore be preferable initially to investigate such correlations in animals which display such predictable behaviour patterns, and especially such patterns as are inborn and therefore relatively independent of environmental conditions. Arthropods, and particularly insects, are suitable for such an investigation, and fortunately there is an abundant literature on the physiology of these animals.

The present work has therefore been mainly an attempt to design

experiments which permit an assessment of the extent to which changes in metabolic rates can be responsible for qualitatively altering behaviour patterns. Attempts have also been made to discover whether, under normal conditions, there is a relationship between metabolic level and the behaviour pattern displayed, and also to what extent the metabolic rate needs to be experimentally altered to produce a qualitative difference in behaviour. The thesis is also concerned with reporting the results of work on certain mechanical and chemical aspects of silk production, which was necessary to help clarify the observations made in the course of the behavioural experiments.

The results presented in this thesis are based on studies of a number of species of spiders and silkworms. The species of spider studied include Zygiella x-notata (Figure 3), Araneus diadematus (Figure 4), Tegenaria domestica, Meta segmentata, Ciniflo similis and Heteropoda venatoria. All these species were obtained in and around Edinburgh. The orb-web spiders were kept in cardboard boxes, mostly 4" x 8" x 11", which had cellophane windows let into the lid and base. The boxes were suspended from a string grid which allowed them to hang in rows of four, so that the light from the window shone directly through the cellophane windows (Figure 5). This arrangement allowed the spiders to spin freely, and it was possible to manipulate the box without either affecting the web or disturbing the spider. The other spiders were kept in tubes or jars, either over moist sand or cotton wool. All the spiders were fed with Drosophila spp., supplied by courtesy of the Institute of Animal Genetics, and the larger spiders were also given blow-flies.

Figure 3. Zygiella x-notata, adult female, dorsal view. (x3 approx.)

Figure 4. Araneus diadematus, adult female, dorsal view (x2 approx.)

Figure 5. Cardboard boxes fitted with cellophane windows in which the orb-web spiders were kept. The boxes were suspended from a string grid, each one containing a single spider.

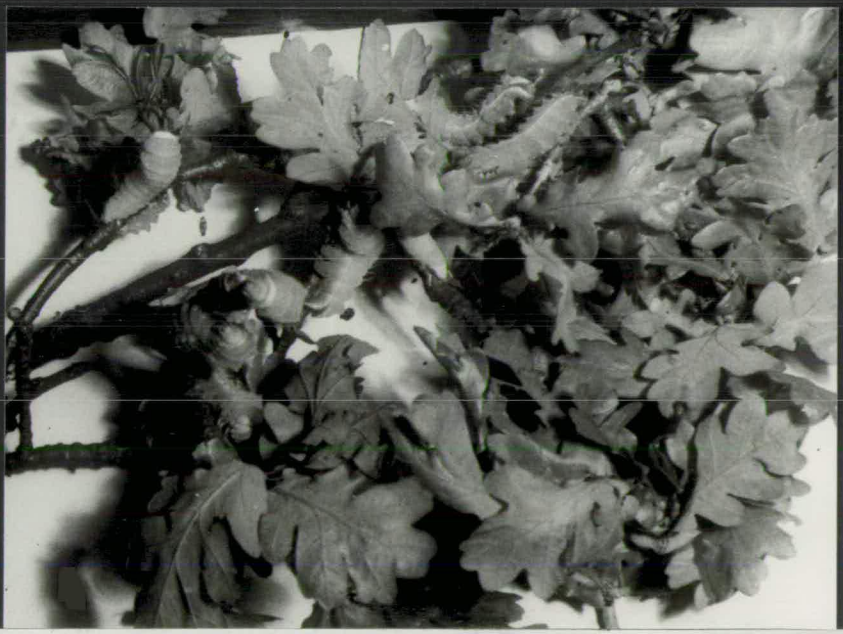

Figure 6. *Antheraea yamamai* larvae, feeding on oak-leaves. (x1/3 approx.)

The species of silkworm studied include Philosamia cynthia, Antheraea yamamai (Figure 6), Bombyx mori, Telea polyphemus and Platysamia cecropia, but most of the experiments were performed on P.cynthia. Professor Carrol Williams of the Biological Laboratory, Harvard University, kindly supplied eggs of the Cecropia silkworm for use in this work. The rest of the silkworm material was obtained from the Butterfly Farm, Bexley Heath, Kent. The stocks of larvae were reared in glass or polythene containers at 25°C., while individuals selected for experiment were kept separately in 4" x 1¼" glass vials. Since silkworms are very prone to disease, a great deal of care had to be exercised in keeping the stocks of these animals healthy.

SILKWORMS AND SPIDERS AS MEDIA FOR BEHAVIOURAL STUDIES

Throughout the five instars of the silkworm, periods of feeding alternate with periods of moulting, and the larval stage of this animal can be looked on as being specialised for rapid growth, whereas the adult is specialised for reproduction.

The sequence of events which characterise the onset of metamorphosis are well known: the larva firstly stops feeding, and evacuates its gut; it then wanders about, apparently at random, finally "choosing" a place where it settles down and spins its cocoon (Figures 7&8). When the cocoon is finished, the larva enters the prepupal stage and finally pupates. The form of cocoon spun by any particular species of silkworm depends more on its inherited pattern of spinning behaviour than on the environmental conditions at the time of spinning, and the limiting conditions for normal cocoon spinning have been exhaustively investigated by Yokoyama (1951) and Van der Kloot and Williams (1953a).

Although the cocoon is the most obvious structure spun by the silkworm, the onset of metamorphosis is not the only time when spinning occurs. Just before each moult it is found that the larva spins a small sheet of silk known as the "moulting pad". This is gripped by the prolegs, and serves as an attachment during the moulting period.

Clearly there is a relationship between the spinning of the moulting pad at the larval moult and cocoon at metamorphosis. In each case spinning occurs during a period of metabolic upheaval, and takes place after feeding has stopped. Thus, although the structure of the cocoon is far more complex than that of the moulting pad, and takes much longer to complete, it would be difficult to deny

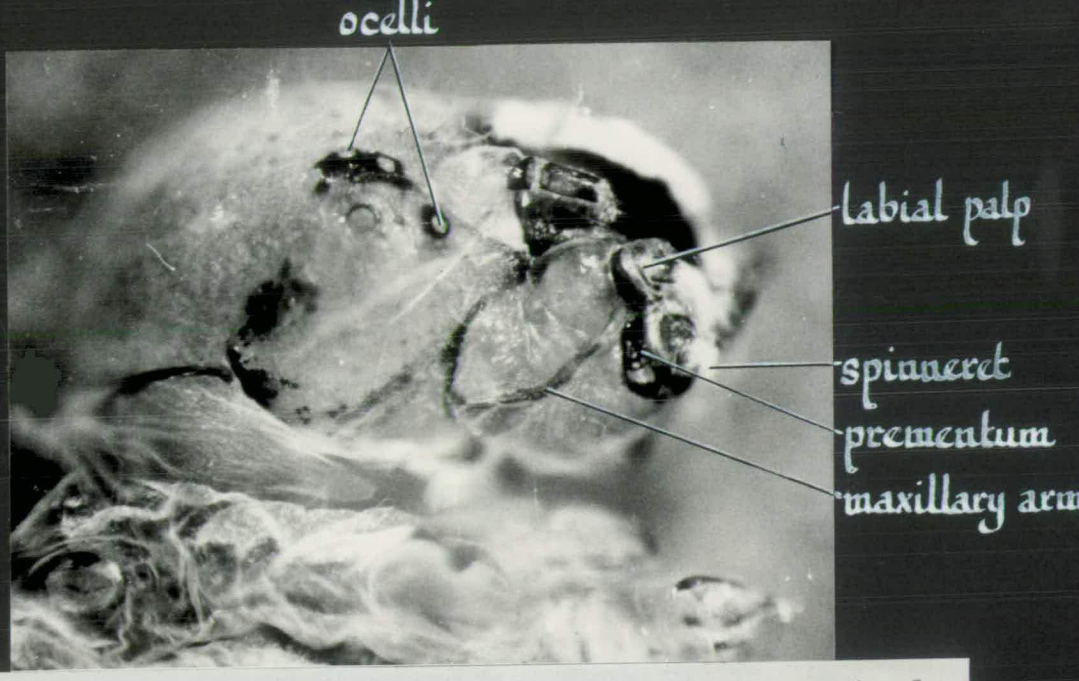

Figure 7. Head of a Cynthia larva seen in ventral view, showing the spinning apparatus. (x14 approx.)

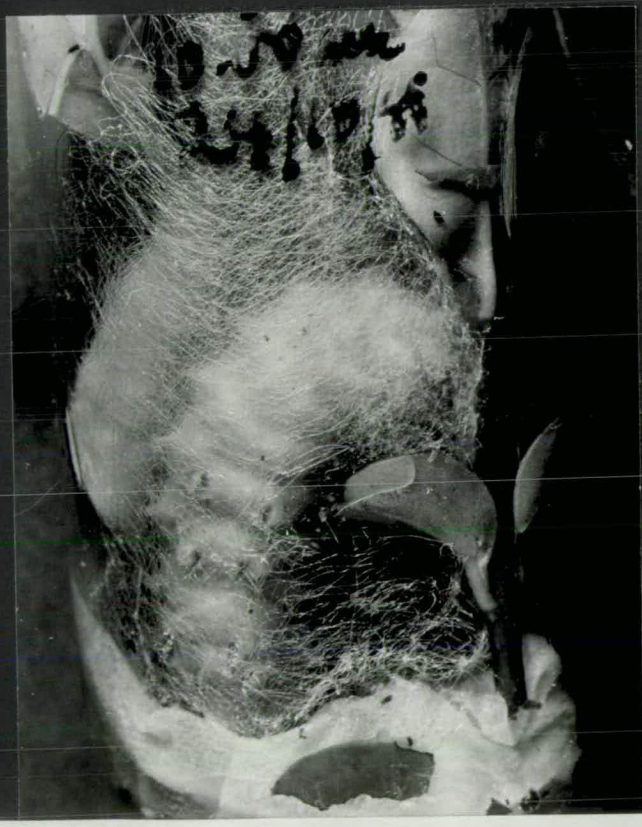

Figure 8. Cynthia larva spinning a sheet of silk on the side of a glass vial. (x2 approx.)

the possibility that spinning in both cases is initiated by the same set of factors.

In investigating the factors which initiate spinning behaviour by their direct effect on the brain, it is necessary to study the internal environment of the animal, and evaluate which of the physiological changes associated with moulting and metamorphosis could be responsible for the observed effects. Many workers have used the silkworm as an experimental animal, and consequently there is an extensive literature dealing with its physiology, particularly with reference to metamorphosis, and the associated endocrine changes. The silkworm, therefore, offers many advantages for use in experiments designed to investigate the link between behaviour and the underlying physiology, and it may be that the results of such experiments could suggest principles which might help to elucidate further problems of a similar nature.

Young orb-web spiders spin perfect webs before they have had any opportunity to learn. These webs are relatively smaller than those spun by the adult, and have fewer radii, but they remain essentially similar. Normally a fresh web is spun once a day, generally in the evening or early morning, and the literature contains many good accounts of this process (Savory, 1952: Bristowe, 1958). The orb-web is very complex, and its structure accurately mirrors the movements of the spider which spins it, Like the silkworm's cocoon, therefore, it is suitable for use in the experimental investigation of behaviour. One cannot, however, suppose that the spinning of the web is linked to some gross metabolic change, since it is part of the normal day to day life of the spider. The initiation of web-spinning is more likely to depend on some diurnal activity rhythm, possibly a light/dark periodicity.

It is interesting to note that, although juvenile males spin normal webs, they lose this power at the last moult when they become sexually mature. At this time their whole behaviour alters as they set off in search of a mate. This change in behaviour of the male is a deep-seated one, and is linked to gross physiological changes, which include the maturation of the gonads, and the loss of two types of silk glands - those concerned with the production of silk for the viscid spiral (Sekiguchi, 1955b).

This is not the only example of a spider abandoning web-spinning, since it occurs also in both males and females of the genus Pachygnatha. These are spiders of the orb-web group which have returned to the hunting mode of life, and it is therefore interesting to find that the very young specimens still build typical orb-webs (Balogh, 1934). Little is known, however, about their silk glands, or whether this behavioural change occurs at a moult.

Lastly it may be mentioned that a less spectacular, but nevertheless important, change in behaviour occurs in juvenile spiders just before each moult. Regular web-spinning stops, and the spider creeps away to hide until moulting has been completed.

With spiders, therefore, it is in connection with these major behavioural changes that a study of web-spinning might throw light on the relationship between physiology and behaviour.

Spiders and silkworms offer unique advantages for behavioural experimentation, and their spinning behaviour provides valuable evidence in evaluating the effect a particular experiment may have had on their behaviour. For this reason these animals are particularly suitable for use in an investigation into the physiological control of behaviour.

EXPERIMENTAL APPROACHES IN PHYSIOLOGICAL STUDIES OF BEHAVIOUR

In recent years there have been attempts to estimate the effects on silk spinning in arthropods by interfering with the animals' physiological balance. These have mainly involved brain surgery techniques, the use of neuronic drugs, and gland extirpation techniques. The experiments of Van der Kloot and Williams (1954) on the effect of certain brain operations on cocoon spinning by the Cecropia silkworm, only served, in their estimation, to establish that spinning is controlled by the brain. They found that accurate mid-line transection of the brain had little effect on cocoon spinning, whereas transection through one or both hemispheres, resulted in the larva spinning a flat sheet of silk consisting of one or two layers. If both circum-oesophageal commissures were cut, spinning did not occur. Electrocoagulation of localised regions of the brain demonstrated that the corpora pedunculata played an important part in the regulation of normal spinning. These authors also showed that transection of the ventral nerve cord at various levels interfered with locomotion, but did not seem to affect the spinning behaviour. In those surgical experiments, however, which resulted in the production of aberrant structures, locomotion appeared to be well co-ordinated.

Experiments of this kind have not up till now been carried out on spiders. The reason for this would seem to be that these animals are not very suitable for surgical operations, since the continuously maintained pressure of the body fluid is such, that only a small puncture can be tolerated by the animal without it suffering from excessive bleeding. Moreover, the animal is rendered incapable of normal movement if the pressure of the body fluid is reduced,

because the mechanism of leg extension depends on the animal's turgid state (Ellis, 1944: Parry, 1957). The aggregation of the ganglia into a compact mass lying centrally in the prosoma also increases the difficulty of establishing a precise operational technique.

Brain surgery experiments, although capable of producing interesting effects, would not therefore appear to be a suitable means of assessing the dependence of spinning behaviour on the underlying physiological processes. Great advances have recently been made in the techniques of electrical stimulation, recording and destruction of brain tissues, by means of micro-electrodes. These techniques, however, have been developed with especial reference to the vertebrate brain, and their successful application to the insect and arachnid brains is hindered by the small size of the ganglia in these animals. Moreover, the brain is situated on the top of the oesophagus, surrounded by the powerful jaw muscles, and moves up and down whenever food is masticated or swallowed. In consequence of this it is very difficult to pin-point actual groups of cells, and either record from or destroy these, without the accidental involvement of neighbouring areas of tissue. The arthropod brain is an exceptionally compact structure and has hitherto defied attempts at analysis by neurophysiological methods such as are regularly practised on the brains of vertebrates. It seems then that techniques involving surgical operation on the brain must be abandoned for the time being, in experiments designed to investigate the physiological basis of arthropod behaviour.

Another line of approach, which has been used for investigating spinning behaviour, is that of administering various drugs, which

are known to have an effect either on the insect or vertebrate nervous system. The orb-web spider, Zygiella (=Zilla) x-notata, has recently been the subject of extensive pharmacological experiments carried out by Witt (1956). He studied the normal web, and then analysed the various malformations resulting from treatment of the spiders with a wide range of neuronic drugs. He claims that each of these produces a characteristic defect in the web, and submits photographs of the webs spun by spiders treated with different drugs to illustrate these defects. It is interesting to note that the defects are in the form and pattern of the web, and not in the omission of a specific part, such as the viscid spiral.

The author did not, however, attempt to evaluate his result from a behavioural standpoint, but it is clear that his evidence strongly supports the idea that specific changes can be produced in the brain by specific chemical agents, or in other words, that chemical factors in the blood could control behaviour by direct action on the brain.

Spinning behaviour in the silkworm has also been investigated from this angle, and Van der Kloot and Williams (1954) have studied the effects of strychnine, atropine, pilocarpine and malononitrile on cocoon spinning by Cecropia. Strychnine sulphate and pilocarpine hydrochloride, at final concentrations of 4×10^{-5}M. and 2×10^{-3}M. respectively, eliminated spinning. At lower concentrations, however, the same agents neither interfered with the spinning behaviour nor injured the animals. Atropine sulphate, at final concentrations of 2×10^{-2}M. or over, was lethal, but at 2×10^{-4}M. the treated larvae spun only flat sheets of silk. Malononitrile blocked spinning at

concentrations of 1×10^{-4}M. or higher, while lower concentrations failed to affect the spinning behaviour, except in two cases where the outer envelope was spun as a flat sheet.

Earlier work on the action of chemical agents on the insect nervous system has been reviewed by Roeder (1953), and it would seem that a detailed physiological analysis of the behavioural effects of these substances cannot be attempted, until far more is known about the functional morphology and biochemistry of the arthropod brain.

A great deal of attention has also been directed to upsetting the hormonal balance in arthropods, in order to study the effects of such treatment on growth and differentiation. It is now well known that moulting and metamorphosis in insects is controlled by the concentration of the "growth and differentiation" hormone (or "moulting" or "pupation" hormone) and the "juvenile" hormone, secreted by the prothoracic glands (Figure 9) and the corpora allata (Figure 10) respectively; the whole cycle depending on neurosecretory activity in the brain (Williams, 1952: Wigglesworth, 1954). Spinning in the silkworm is intimately bound up with these processes, and the onset of spinning is therefore ultimately dependent on the hormone balance in the body.

Precocious metamorphosis in the silkworm was first produced by Bounhiol (1937), who removed the corpora allata from 3rd and 4th instar Bombyx mori. These larvae underwent premature maturation, spun cocoons, pupated, and finally emerged as perfect, but small, adults. Fukuda (1951) has shown that a similar effect can be produced by ligation just behind the head, but, although the silk glands

Figure 9. Dissection of a Cynthia larva to show the prothoracic gland. (x25 approx.)

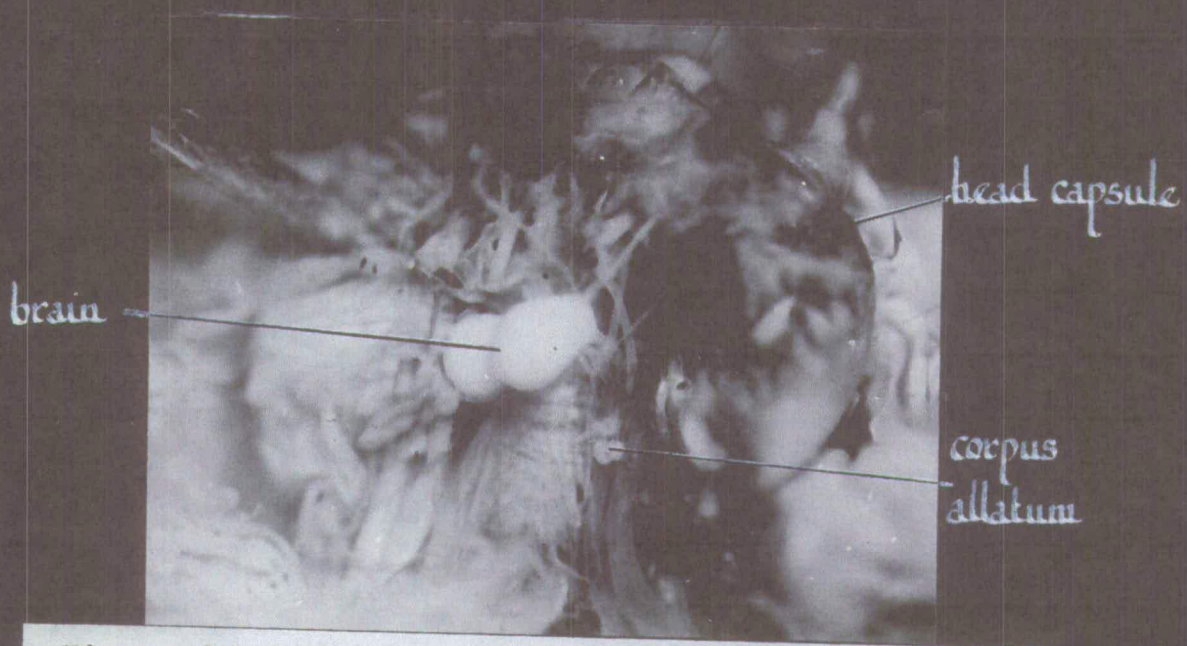

Figure 10. Dissection of the head of a Cynthia larva, showing the brain and corpus allatum of one side. (x25 approx.)

mature normally, this technique prevents the larvae from spinning. These experiments show that the larval brain can direct cocoon spinning at any stage after the 2nd instar, given the correct internal environment. They do not prove, however, that the hormones affect the nervous tissue directly to produce the change in behaviour.

Piepho (1950) studied the spinning of Galleria both under normal conditions, and after the implantation of corpora allata. The structure spun just before a normal moult is a thin-walled, open-ended tunnel, whereas the cocoon is thick-walled and is closed at one end. By implanting various numbers of active corpora allata into last instar larvae, he induced the formation of one or two extra larval stages, but, more commonly, forms were produced intermediate between larva and pupa. The structures spun by these intermediate forms were also intermediate between the open-ended tunnel and the cocoon. He concluded that the hormone from the corpora allata influenced the type of spinning by acting directly through the nervous system.

It is possible, therefore, that the brain is directly affected by the hormones responsible for moulting and metamorphosis, and that spinning will take place only under suitable hormonal conditions. On the other hand, spinning occurs at a definite point in the sequence of events leading up to pupation, and may well depend on some other factor, operating within the somatic conditions induced by the hormones.

In spiders, the hormonal control of moulting has not received the attention awarded to the same process in insects, but the presence of neurosecretory cells in the brain and ventral ganglion

mass has been demonstrated in various species of spider (Gabe, 1954: Legendre, 1954a, b), and there is little doubt that the moulting cycle is controlled by their secretions (Gabe, 1955). The premoult change of behaviour, when regular web-spinning stops, is therefore probably linked with the hormonal change associated with moulting. Similarly, it might be suggested that both the morphological and behavioural changes occurring in the male orb-web spider at the last moult, are hormonal effects.

We can conclude that the hormone balance in the body plays a very important part in controlling the behaviour of both the silkworm and the spider, but it should be remembered that the evidence does not give any indication as to what factors act directly on the brain so as to produce the observed behavioural changes. The possibility remains, however, that the moulting hormone may influence the brain of the silkworm directly so as to initiate spinning, and that an equivalent process may also occur in the spider, where the effect is, however, to stop spinning. A very marked increase in concentration of moulting hormone occurs at the time when the inner capsule of the cocoon of Cecropia is being spun (Schmidt and Williams, 1953), and the timing of this increase would therefore appear to link the hormone with the cessation, rather than with the initiation, of spinning. Whether the silk glands themselves act as endocrine organs in these animals is still an open question, but hitherto there seems to be little experimental evidence to support this suggestion. It would, however, be of great interest to know precisely how the hormones associated with moulting and metamorphosis control spinning behaviour, but it is clear that such an investigation demands techniques in advance of those, which up till now,

have been responsible for adding a great deal to our knowledge of insect endocrinology.

There is also the outstanding question of what changes take place in the composition and function of the blood of arthropods, during the various well-defined phases in their life-cycles. Millott (1949) states that these are striking changes in the cell composition of the blood of the spider just prior to the moult, the most obvious being the accumulation of vacuolated leucocytes. These occur in such numbers that they occlude the heart completely and fill the haemocoel, making the flow of blood negligible. The blood must be responsible for transporting oxygen from the lung-books to the brain in the orb-web spiders, because the tracheal system in these animals is very reduced, and is restricted to the abdomen. It is therefore possible that the brain lacks an adequate supply of oxygen during this period. This effect might be directly responsible for the changes in behaviour which occur prior to moulting, since anoxia would probably alter nervous thresholds in the brain.

Van der Kloot and Williams (1954) report that Cecropia larvae retained normal muscular co-ordination when subjected to from three to thirty-two pounds per square inch of carbon monoxide, but were unable to spin normal cocoons. When returned to air, however, they spun normally with their remaining silk. In another experiment, in which larvae were subjected to low oxygen tensions, one larva spun a sheet of silk while in a 3% oxygen: 97% nitrogen mixture, but again spun normally when returned to air. At all oxygen tensions higher than this, the larvae were unable to spin normal cocoons.

The results of this particular work are in accord with the possibility already indicated, that patterns of behaviour may be more sensitive than muscular co-ordination, to treatment which directly affects respiratory metabolism. These results also point to the possibility that changes in brain metabolism, whether due to anoxia or even to a change in overall metabolism, might be able to account for changes in behaviour.

SILK SPINNING IN SPIDERS

Despite the wealth of information available on silk spinning by spiders, several questions remained unanswered concerning the principles involved in silk production.

The arrangement and form of the various spinning organs and silk glands had been described for many families (Apstein, 1889: Hopfmann, 1935: Savory, 1952: Sekiguchi, 1952, 1955a, b), but there was still some uncertainty as to the function of the duct in the transformation of the silk substance into the thread which emerges from the spinneret. Neither was it clear whether the body pressure played any part in the movement of silk down the duct, or helped to maintain the silk right up to the orifice of the duct, as a prerequisite for enabling the animal to pull the silk out as a thread.

It has been conclusively shown, however, that the actual formation of the silk thread depends on the orientation of the molecules under tension, the process being one of crystallisation (Ho *et al.*, 1944: Savory, 1952: Kendrew, 1954). It is therefore necessary for the silk to the pulled, and not squeezed, out, and this obviously presupposes the prior attachment of the silk to some solid object. On the other hand, the silk may be pulled out by the legs or by air currents, as in the case of "gossamer" silk. Since the spider has several different types of silk available, in each case where a particular type is required, the movements of the body and spinning apparatus must be such that the correct type is extracted, and this aspect of silk production has also remained rather obscure.

It was therefore essential to re-examine the spinning apparatus, and design experiments to permit an estimation of the extent to which silk production is a purely mechanical process. To gain insight into this problem, it seemed that an investigation of the production and attachment of the dragline silk might be a rewarding way of approaching the subject, rather than attacking the wider problem of web-spinning directly.

A. The spinning apparatus

In orb-web spiders, the spinning apparatus consists of a number of silk-secreting glands lying in the ventral half of the abdomen, which communicate with their individual spinning tubes by long, and in some cases coiled, ducts. The spinning tubes are situated on the spinning surfaces of the spinnerets, six in number, which lie grouped on the ventral surface of the abdomen together with the colulus and the anal papilla.

1. The structure of the glands, spigots and spinnerets.

This investigation of the glands and spinnerets of Zygiella and Araneus was carried out by the usual methods of cutting serial sections 3 to 8 mu. thick, of preparing thick sections and mounts of whole organs, and of dissection. The use of a microscope fitted with a foot-focussing control proved invaluable for dissection and for carrying out the surgical operations described later in this thesis. Bouin was used as the fixative, and the material was embedded in 56°C. melting point paraffin wax. The principal stains used were Mayer's Haemalum, Eosin and Chlorazal Black E.

Shirlastain A, supplied by I.C.I. Ltd., proved to be of great value in microanatomical work because of its speed in staining, and the vivid polychromatic effects produced.

The position of the spinnerets on the abdomen of Zygiella is illustrated in Figure 11, while closer views are shown in Figures 12 and 13. It is clear that the arrangement of the large and small spinning tubes, known as spigots and spools respectively, is not a haphazard one. Each bears a definite relationship to the others, and this relationship has a definite bearing on the production of the individual types of silk.

In the adult female orb-web spider, the silk glands are of six types. Dragline silk is produced from a pair of major ampullaceal glands, and consists of two strands of silk coming from spigots situated one on each of the anterior spinnerets. The duct of this gland is characterised by the presence of a prominent loop, which increases its length about two and a half times. These features are illustrated in Figure 14 which shows the gland and duct, and Figure 15 which shows the spigot.

Sometimes the dragline is strengthened by the addition of silk from a pair of minor ampullaceal glands, which open at spigots on the median spinnerets. The duct of these glands is again characterised by a loop, and the spigot is similar in shape to that of the major "dragline" gland (Figure 16).

The cylindrical glands produce silk which is used in the construction of the cocoon which houses the eggs of the spider. There are three pairs of these glands, one pair opening on the median spinnerets (Figure 17), and two pairs opening on the posterior spinnerets (Figures 18 and 19). These glands are remarkable in

Figure 11. Zygiella, ventral view showing the position of the spinnerets on the abdomen. (x3 approx.)

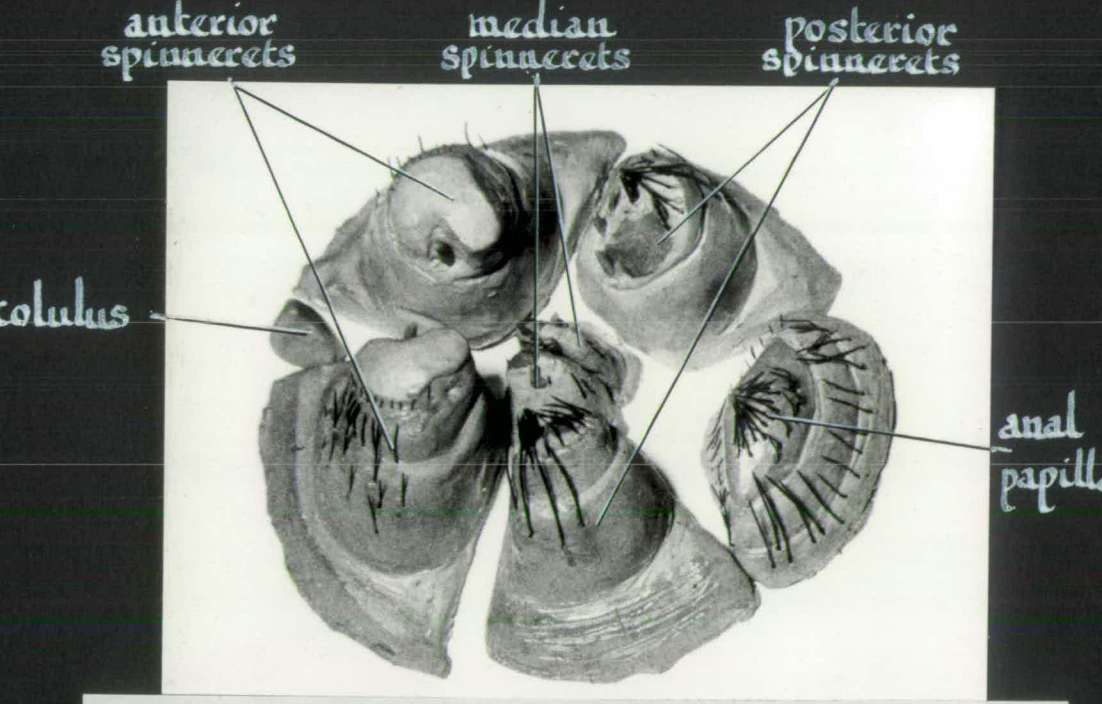

Figure 12. Zygiella, plasticine model of the spinnerets.

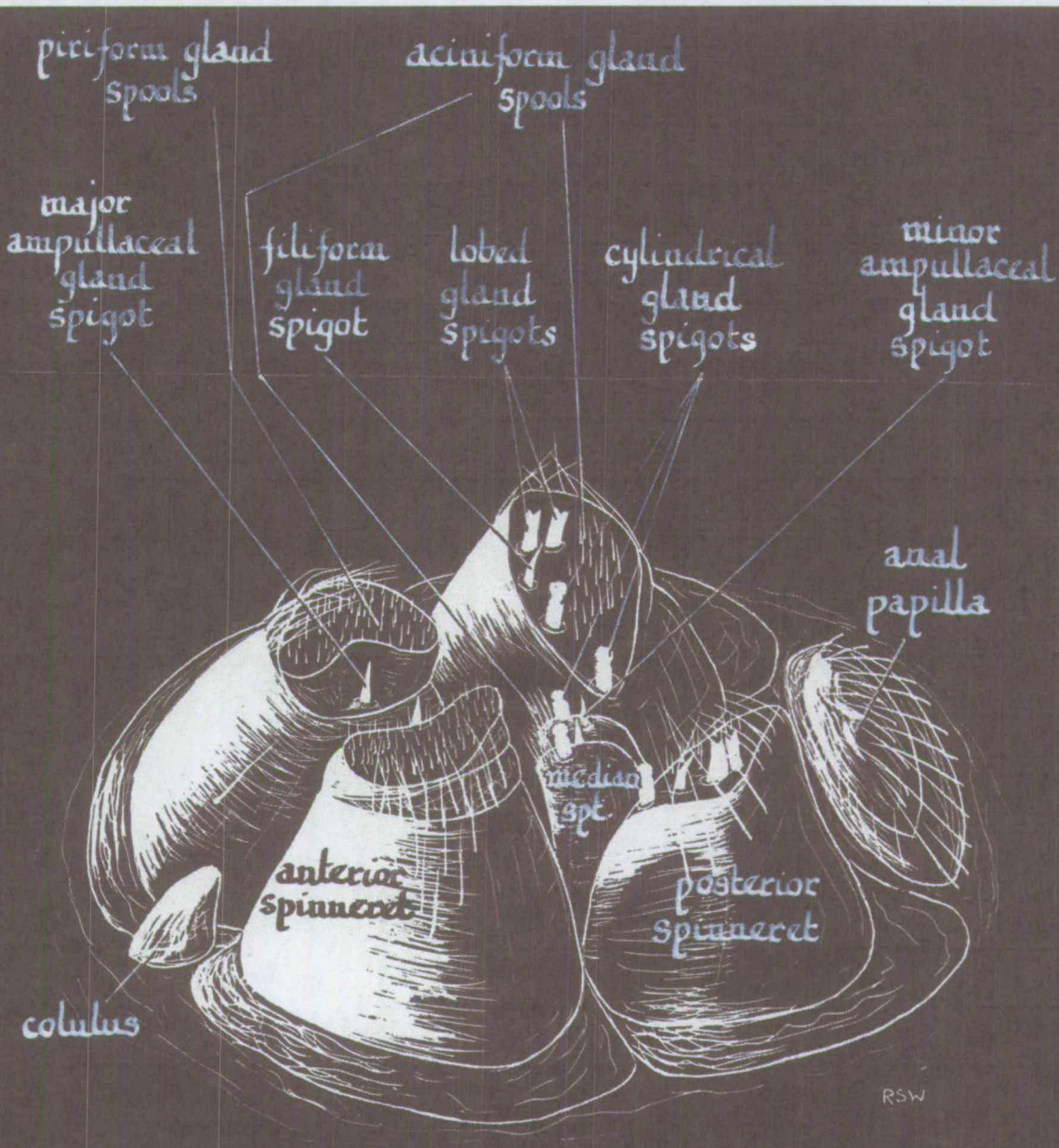

Figure 13. Zygiella, drawing of the spinnerets showing the arrangement of the spigots and spools.

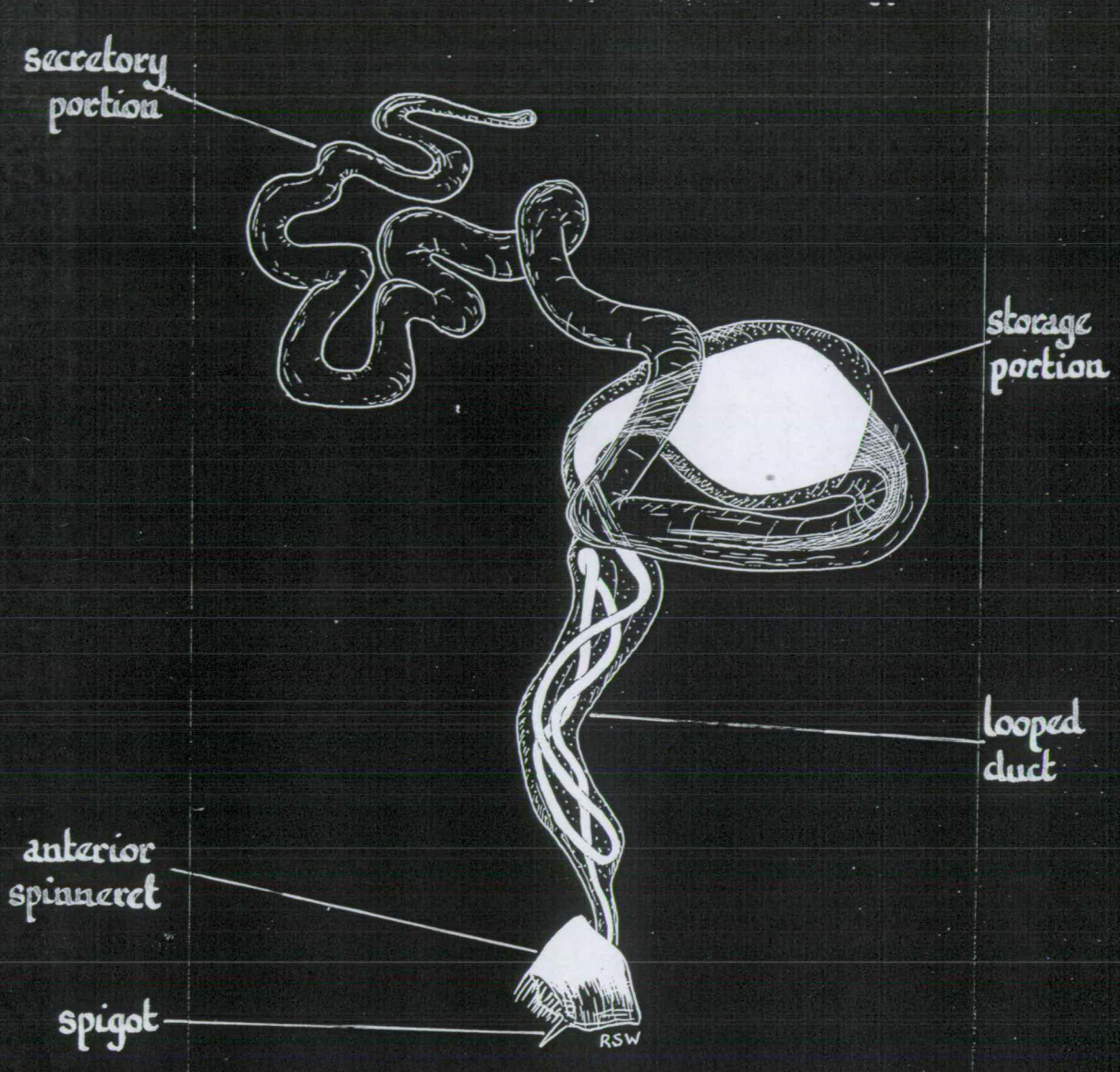

Figure 14. Zygiella, drawing of the major ampullaceal gland and duct, leading to the anterior spinneret.

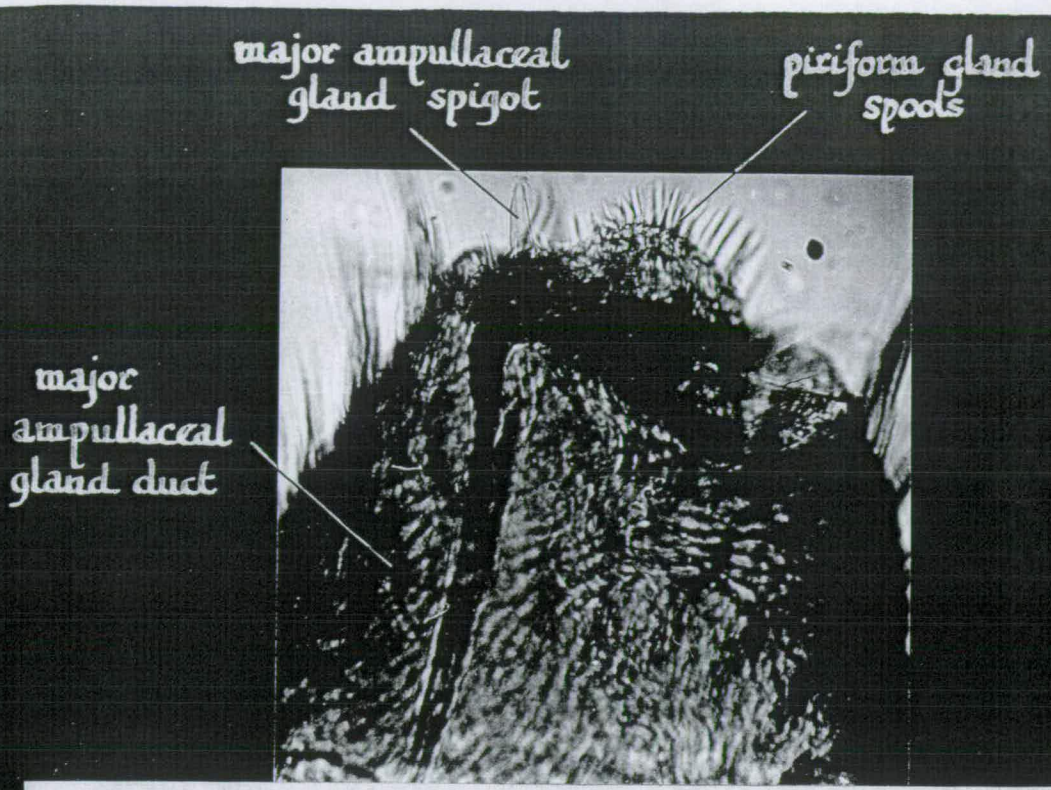

Figure 15. Zygiella, anterior spinneret showing the major ampullaceal gland spigot and duct, also the piriform gland spools. (x 65 approx.)

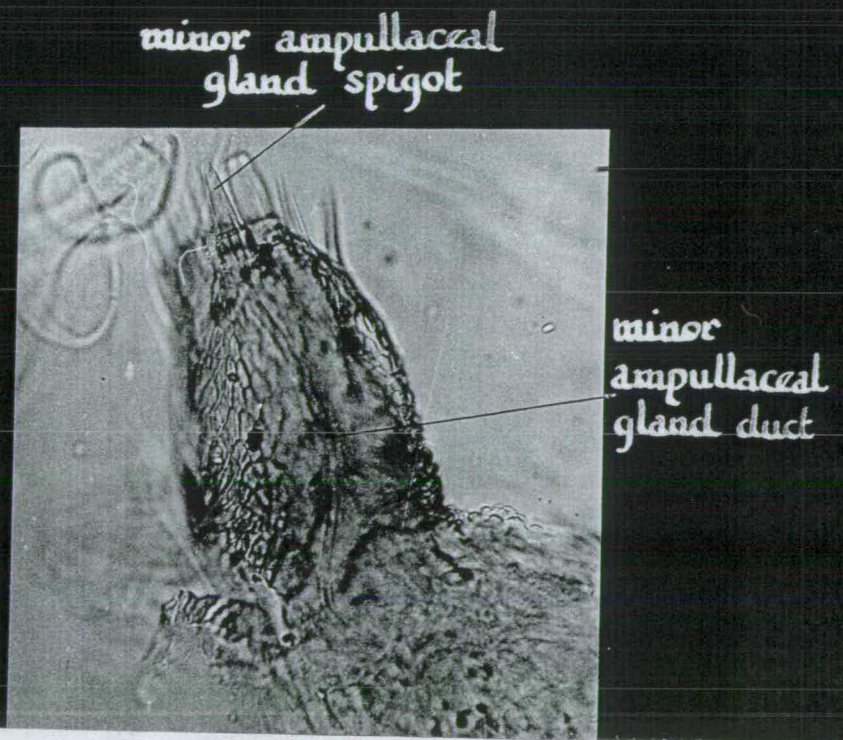

Figure 16. Zygiella, median spinneret showing the minor ampullaceal gland spigot and duct. (x65 approx.)

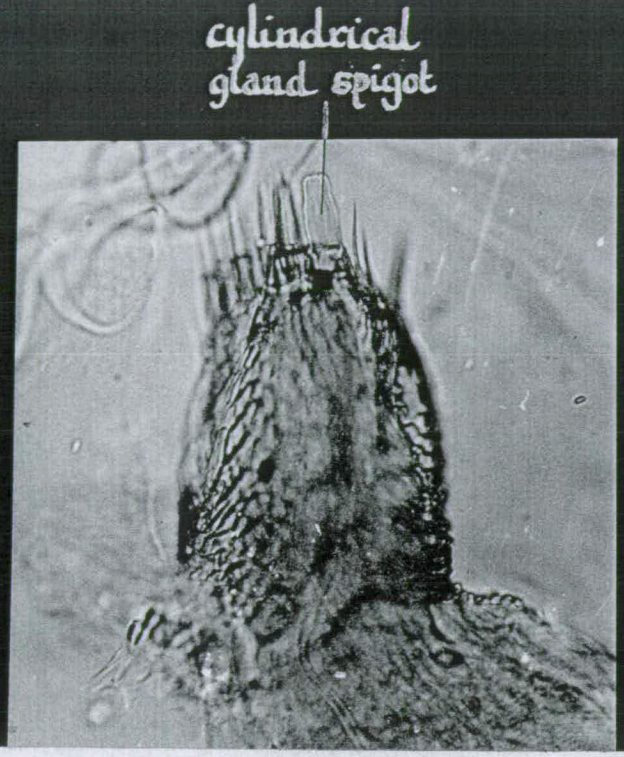

Figure 17. Zygiella, median spinneret showing the cylindrical gland spigot. (x 65 approx.)

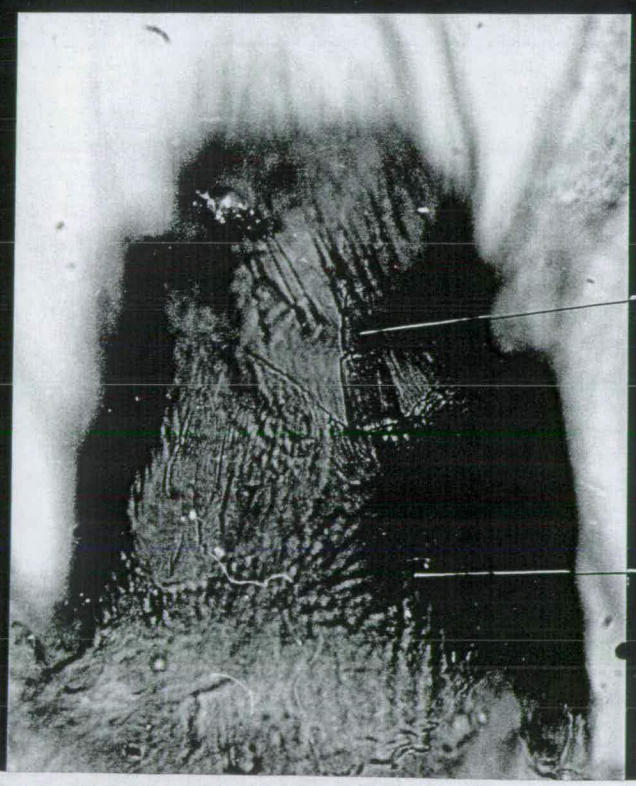

Figure 18. Zygiella, posterior spinneret showing the first cylindrical gland spigot and duct. (x 65 approx.)

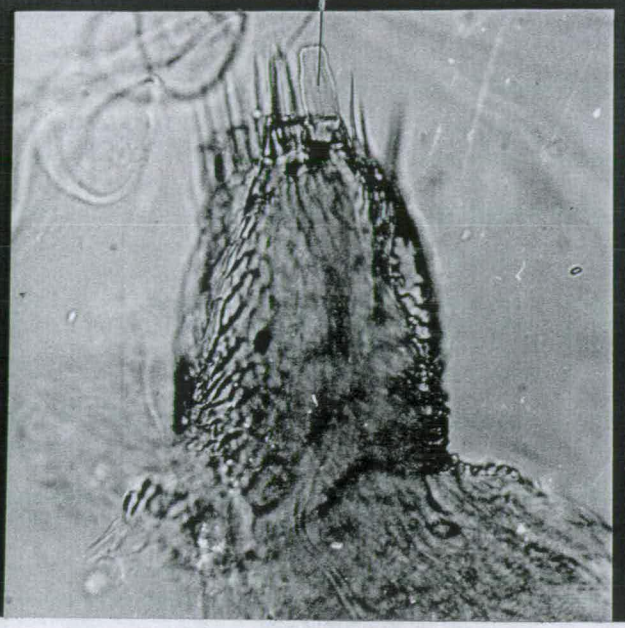

Figure 17. Zygiella, median spinneret showing the cylindrical gland spigot. (x 65 approx.)

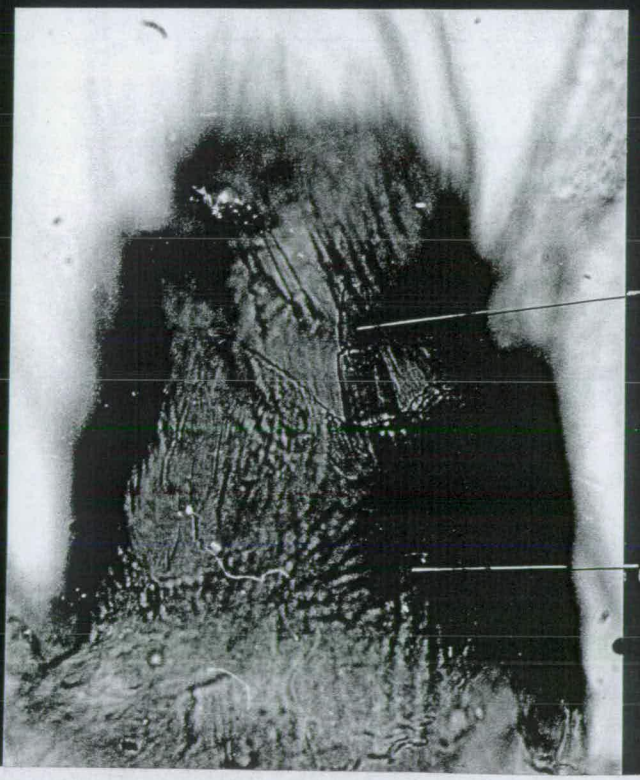

Figure 18. Zygiella, posterior spinneret showing the first cylindrical gland spigot and duct. (x 65 approx.)

having a cycle of silk secretion which follows the growth of the ovaries, and thus differ from the other types of gland which secrete silk continuously (Savory, 1952). The form of spigot with an inturned lip is also characteristic of the cylindrical glands.

It has already been mentioned that two types of gland are concerned in the production of the viscid spiral of the orb-web. These are the filiform or coronate glands which secrete the core, and the lobed or aggregate glands which secrete the sticky substance which gives this particular silk its peculiar adhesive properties. The pair of filiform glands open at spigots very similar to those of the dragline glands (Figure 20), but the two pairs of lobed glands possess spigots which are relatively large in diameter, and have a notched orifice (Figures 21 and 22). The latter glands also have characteristically lobulated ducts.

Associated with the major dragline glands are the smaller and more numerous piriform glands, which open at spools on the top of the anterior spinnerets (Figure 15), and secrete thin, sticky silk used in the formation of the "attachment discs", which will be described below. The aciniform glands open at spools on the median and posterior spinnerets (Figures 17 and 19) where they may play a role in the attachment of the other silks. It is also possible that the "gossamer" silk comes from aciniform glands.

Figure 23 shows the glands from one side of the body of Araneus, displayed after being dissected out and disentangled from the branches of the digestive gland.

Figure 21. Zygiella, posterior spinneret showing the first lobed gland spigot and duct. (x65 approx.)

Figure 22. Zygiella, posterior spinneret showing the second lobed gland spigot and duct. (x65 approx.)

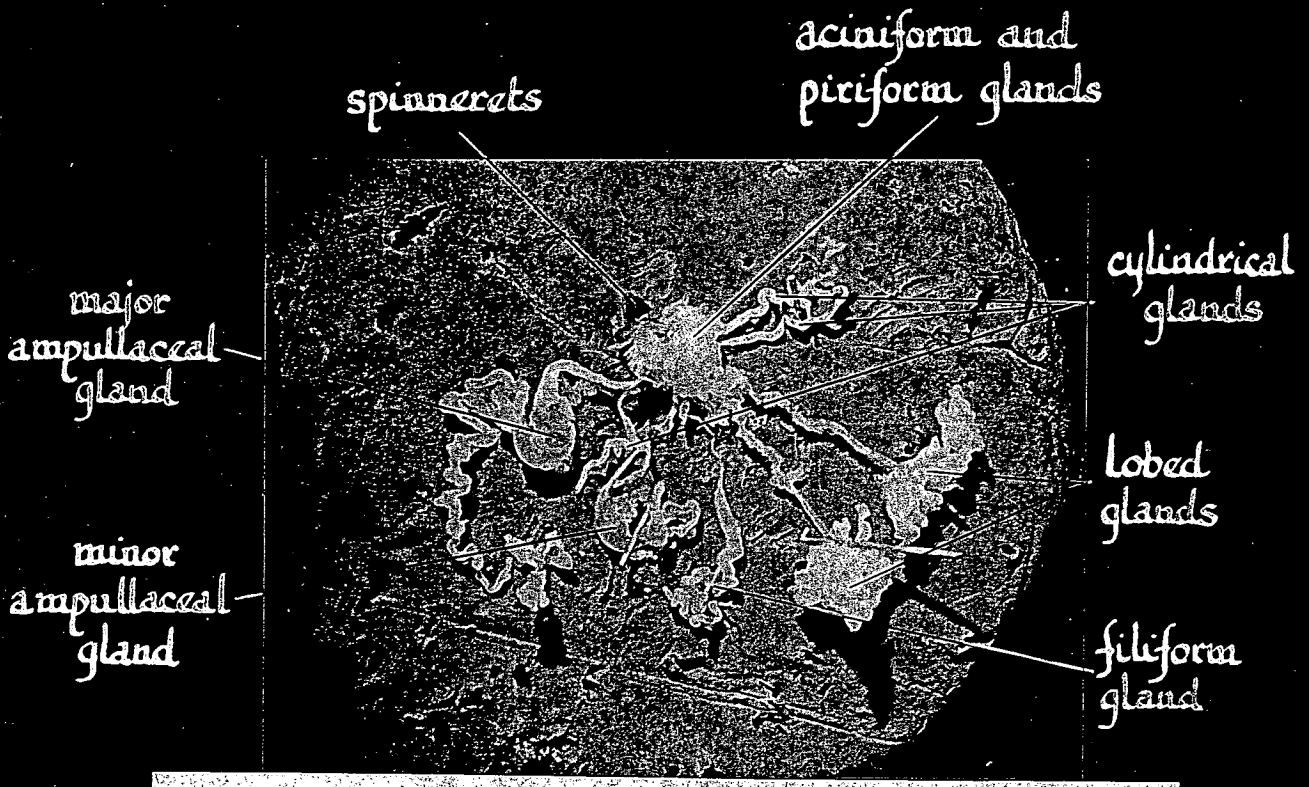

Figure 23. Araneus, silkglands from one side of the body, dissected out and displayed still attached to the spinnerets. (x25 approx.)

2. Production of silk, and the movements of the spinnerets during spinning

Silk is elaborated in the cells of a particular gland, and droplets of the material collect at the proximal ends of the cells and pass bodily into the lumen. These droplets are of different types, as shown by their staining affinities, but they coalesce in the lumen of the gland to form the proteinaceous solution termed silk. The silk produced by one type of gland differs from that of another, but no chemical analysis of these various types has been done. The data on the chemical composition of spider silk given by Savory (1952) probably refers to the average cob-web.

X-ray diffraction studies on the silk of Bombyx (Ho et al., 1944) have revealed that the protein molecules of which the silk is composed, are not arranged in any particular way in the lumen of the gland. The thread as spun by the silkworm, however, gives a characteristic diffraction pattern, which shows that the molecules have been linearly arranged along the length of the thread, during their passage down the duct and out of the spinneret. It is generally accepted that silk formation in the spider depends on a similar process, but it was of interest to know to what extent the arrangement of the molecules depended on the passage down the duct, as opposed to the surface forces operating in conjunction with the loss of water, which would occur when the thread was spun in air. An experiment was therefore devised to test the relative solubilities of silk spun an hour previously, and of that just emerging from the spigot. Various aqueous solutions of detergent were made up, in which anaethetised spiders were submerged after their dragglines had been picked up on a needle. It was found that the strand

disintegrated in the stronger detergent solutions, although it maintained its integrity in the weaker solutions and in pure water. The previously spun silk, however, was unaffected by a short immersion in concentrated detergent, and only dissolved after two days. It seems then that the dragline only achieves its full strength after it has dried out in the air, and that, although the molecules may be arranged by their passage down the duct, it is after they emerge from the spigot that they bond strongly together. This probably occurs very quickly, since the drying of so thin a thread must be exceptionally rapid.

The production of a silk thread involves the manipulation of the spinnerets so that the correct spigots are brought into contact with a solid object, to which the silk is attached, and then the thread is pulled or drawn out as the spider moves away. This process has been studied by direct observation and by filming, in relation to the production and attachment of the dragline, which is used as a "life-line" by the spider, and also forms the non-sticky parts of the orb-web.

The dragline is anchored by an "attachment disc", typical examples of which are shown in Figures 24, 25 and 26. Sticky threads from the piriform glands overlap and fuse round the dragline silk (Figure 27), and also adhere strongly to the surface on which attachment disc is made. Although the general shape of the disc is characteristic of the species studied, the method of construction is very similar in each case. The spider bends down the tip of its abdomen, and moves the anterior spinnerets forward, so that they stick out at right-angles to the body. The tips of the

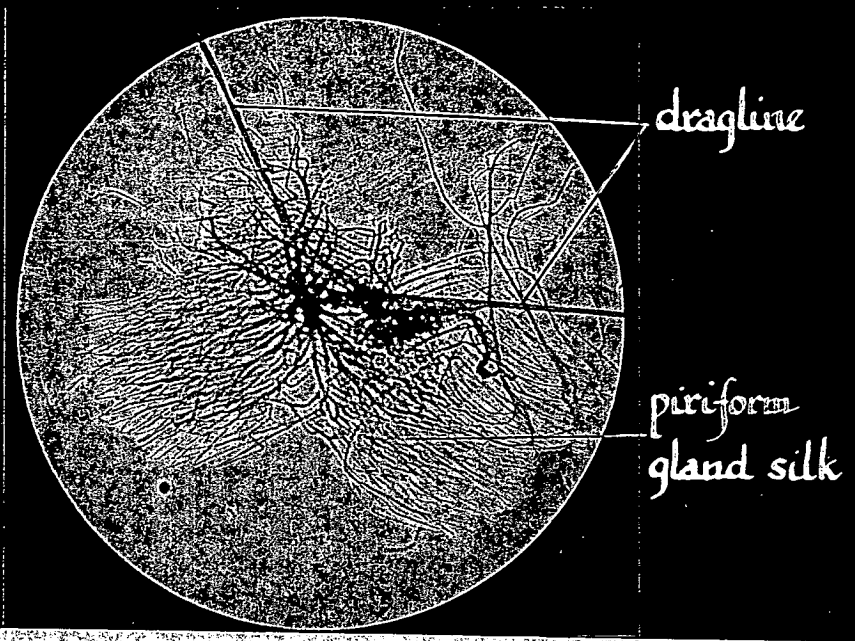

Figure 24. Zygiella, attachment disc. (x50 approx.)

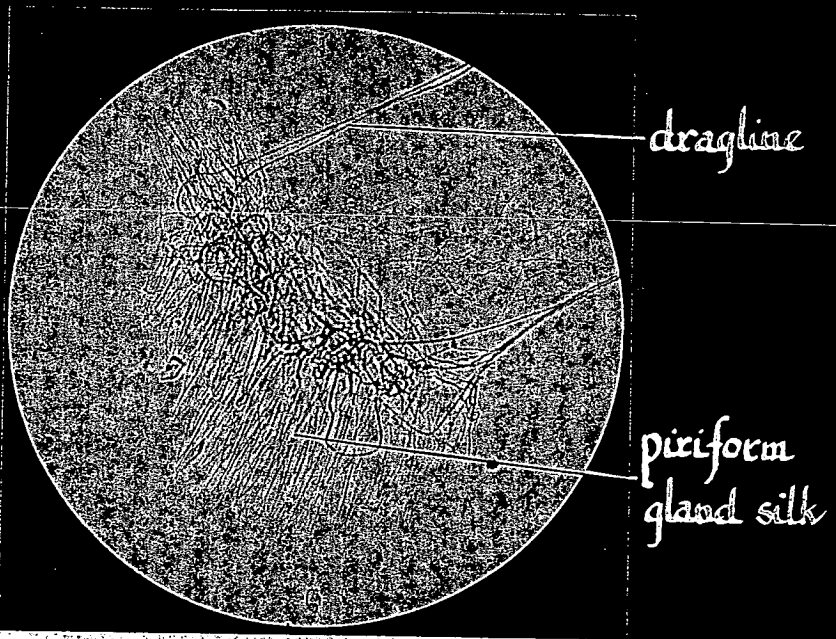

Figure 25. Araneus, attachment disc. (x25 approx.)

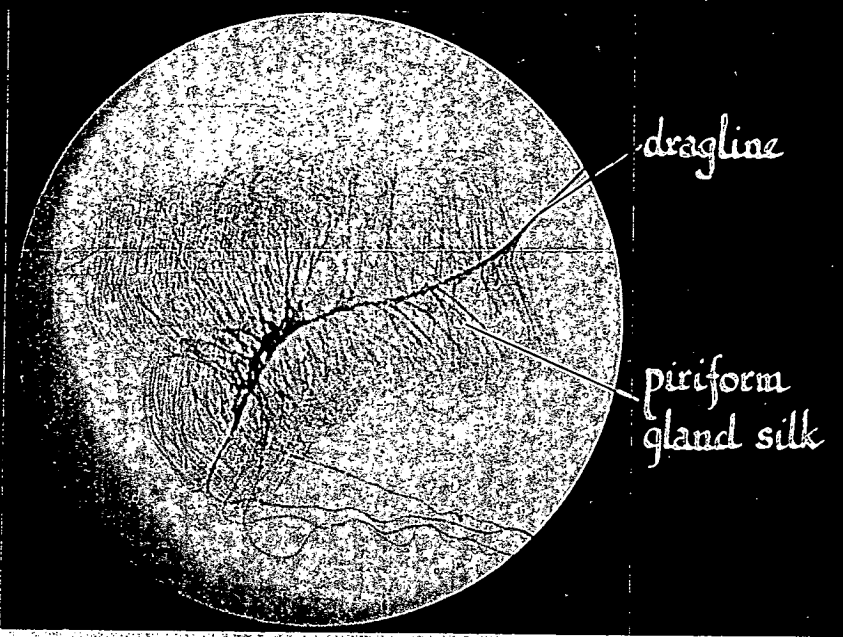

Figure 26. Heteropoda, attachment disc. (x10 approx.)

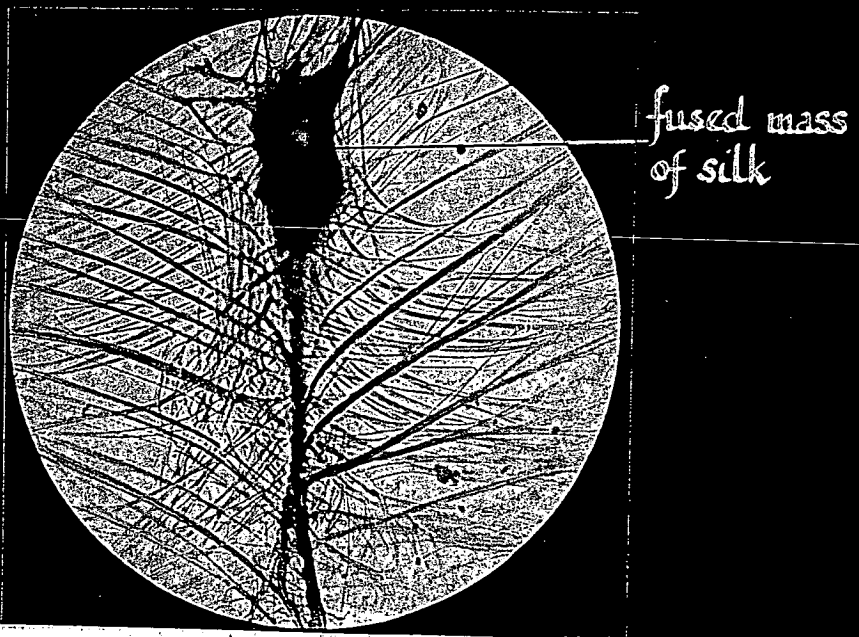

Figure 27. Heteropoda, close-up of attachment disc showing the fusing of the piriform gland silk round the dragline. (x50 approx.)

spinnerets are then brought into contact with the surface, while the spinnerets are moved laterally away from each other and brought back again. By this movement the silk threads from the piriform glands are spread out over the surface, where they may be seen adhering as individual threads, but, as the spinnerets move together again, these strands fuse with each other, and finally coalesce into a lumpy mass round the dragline.

Zygiella characteristically makes attachment discs with only one "in and out" movement of the spinnerets, while Araneus uses several, laid down one in front of the other. Heteropoda, on the other hand, moves its spinnerets in a circular fashion, each one moving alternately, so that a figure-or-eight pattern of threads is laid down as the spider walks slowly forwards.

These movements, in conjunction with the spatial relationships of the spools and spigots, result in the formation of a very efficient attachment for the dragline. It is remarkable that such a complex structure, where the load on the dragline is distributed over the relatively large area of the disc itself, can be formed in such a simple manner.

3. Control valve operation

The fourth pair of legs is normally used by the orb-web spider to manipulate the dragline silk, especially when "dropping on a thread" (Figures 28 and 29). If these legs are amputated, however, the spider can still control or check the rate of its descent, thus indicating that there is some other means of control. This led to the discovery of a valve-like structure in the duct of the

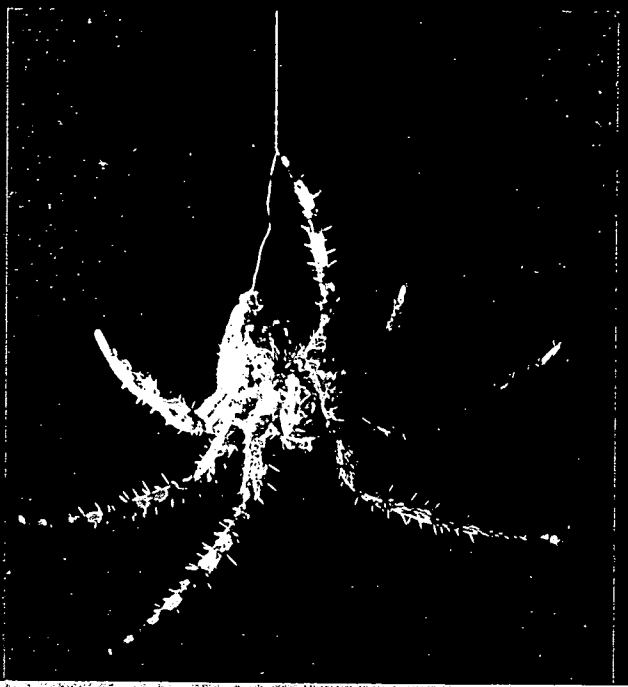

Figure 28. Araneus, hanging suspended on its dragline. The photograph shows the spinnerets infolded and the dragline held by the hind leg. (x2 approx.)

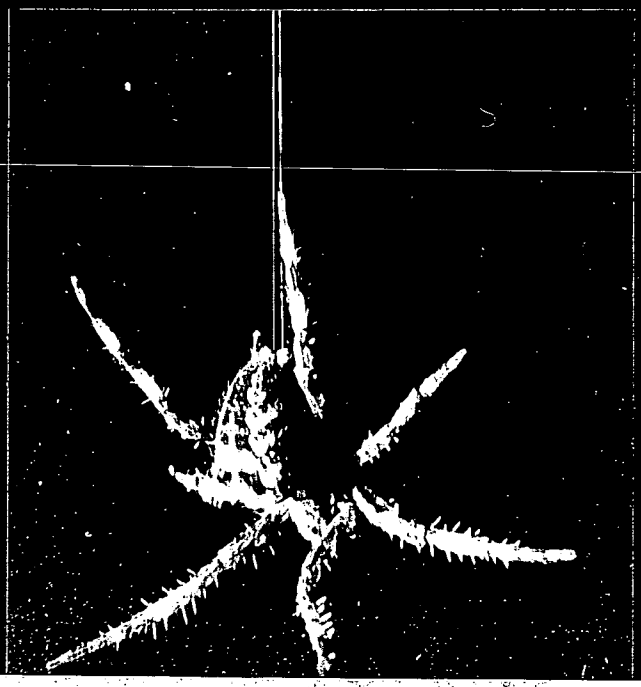

Figure 29. Araneus, dropping on its dragline. The photograph shows two strands of silk emerging from the anterior spinnerets which are held slightly apart. The dragline is sliding through the tarsal claws of the hind leg. (x2 approx.)

major ampullaceal gland. Similar structures were also observed in the ducts of the minor ampullaceal gland and the cylindrical glands, which, however, are of much simpler construction and have no associated musculature.

There is no reference to this control valve in the literature (Schimkewitch, [illegible], 1884: Millot, 1936: Brown, 1939). The valve does not seem to be present in Tegenaria, Ciniflo or Heteropoda, and this suggests that it might represent a specialised feature of the Argyopidae.

The control valve consists of two rounded lips projecting from the cuticular intima into the lumen of the duct, and is found in the region where the duct bends as it enters the base of the anterior spinneret. The lips point in the direction of silk flow, and are connected to a set of muscles by epithelial cells, which have become modified to form tonofibrillae. The arrangement of the muscles and the construction of the valve are shown diagrammatically in Figures 30, 31, and 32. A plasticine model of the valve is illustrated in Figure 33, while Figure 34 is a photograph of a cross-section of the base of the spinneret, showing the valve in longitudinal section.

The muscles associated with the spinnerets can be divided into two groups, "flexors" and "extensors". The flexors are connected to the main bundle of longitudinal muscles in the abdomen, and serve to bend the spinnerets inwards as a group, together with the anal papilla and the colulus. The extensors are inserted at various points in the wall of the abdomen, and serve to spread the spinnerets outwards. The arrangement of these muscles suggests

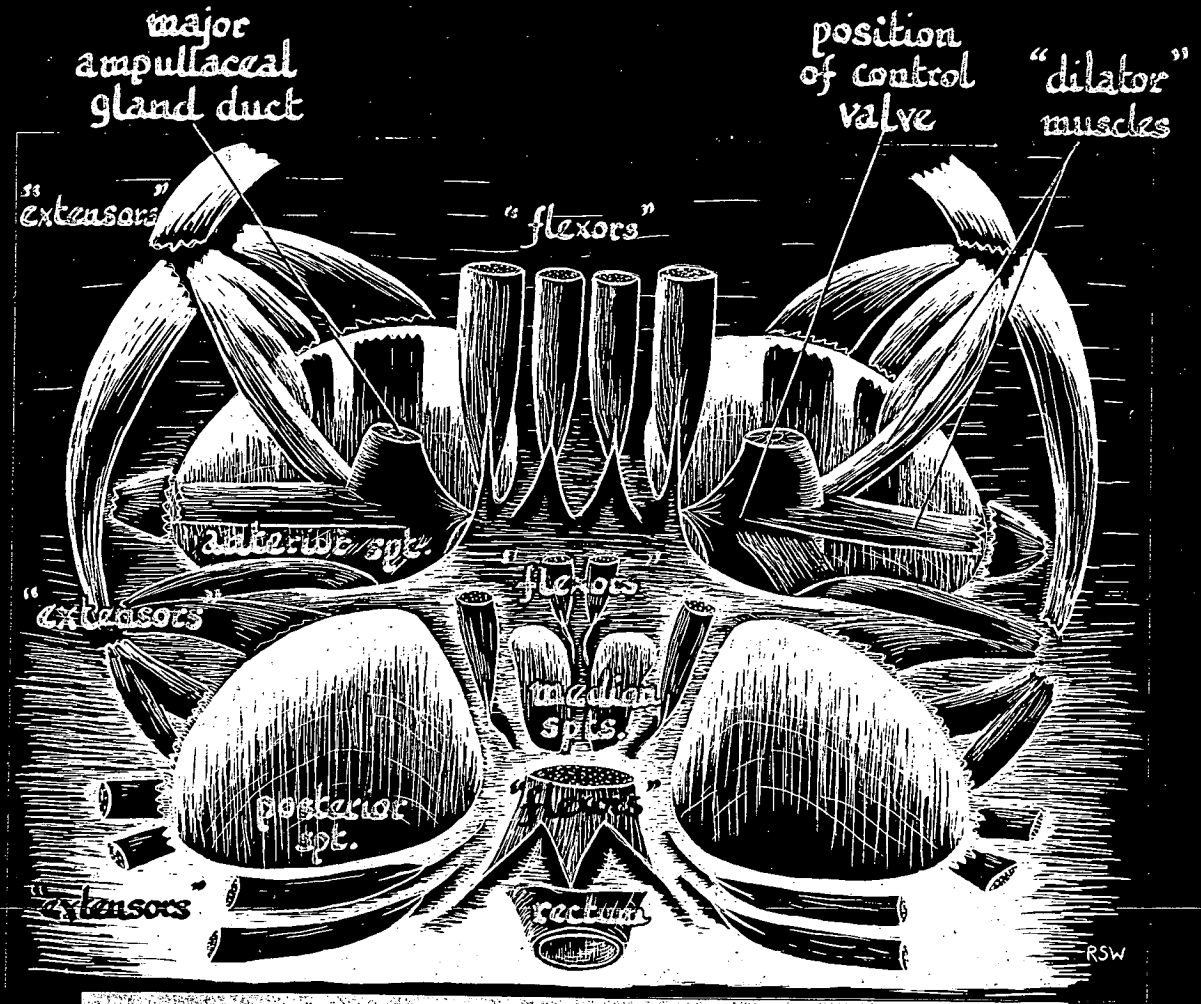

Figure 30. Araneus, schematic drawing of the spinneret musculature as viewed from the inside of the body, showing the position of the control valve in the duct of the major ampullaceal gland.

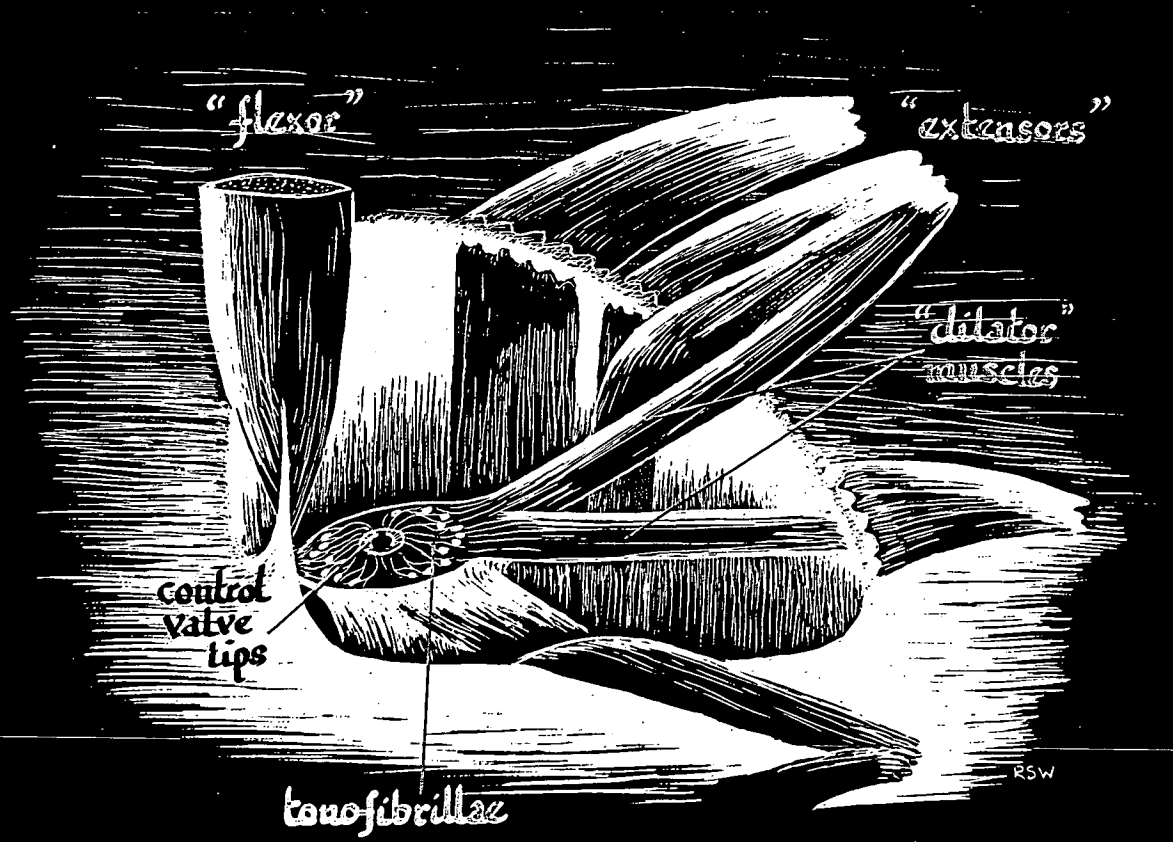

Figure 31. Araneus, schematic drawing of the musculature of the anterior spinneret, showing the control valve in cross-section.

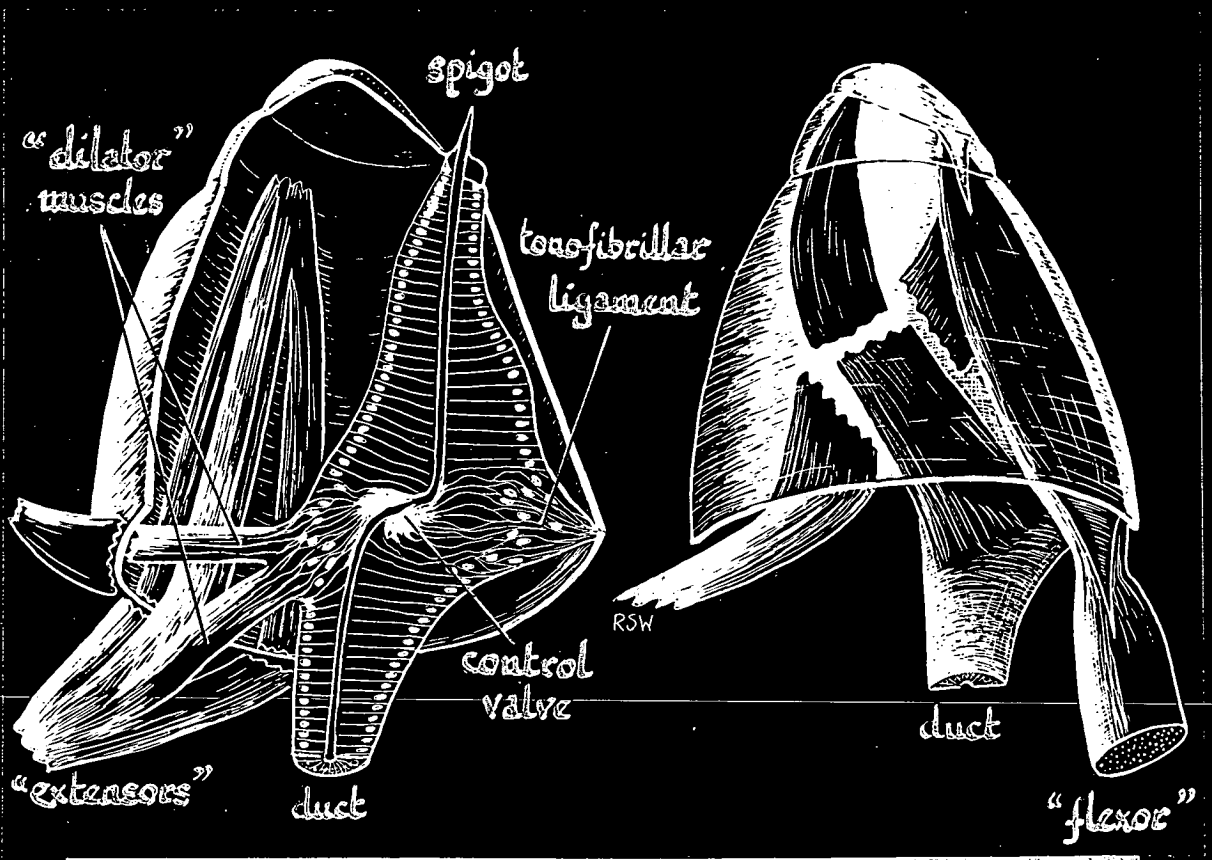

Figure 32. Araneus, schematic drawing of the musculature of the anterior spinneret, showing the spinneret divided longitudinally down the line of the major ampullaceal gland duct. The control valve is shown in longitudinal section, lying in the bend of the duct, and is connected to the spinneret wall by a tonofibrillar ligament, which serves as an antagonist to the dilator muscles.

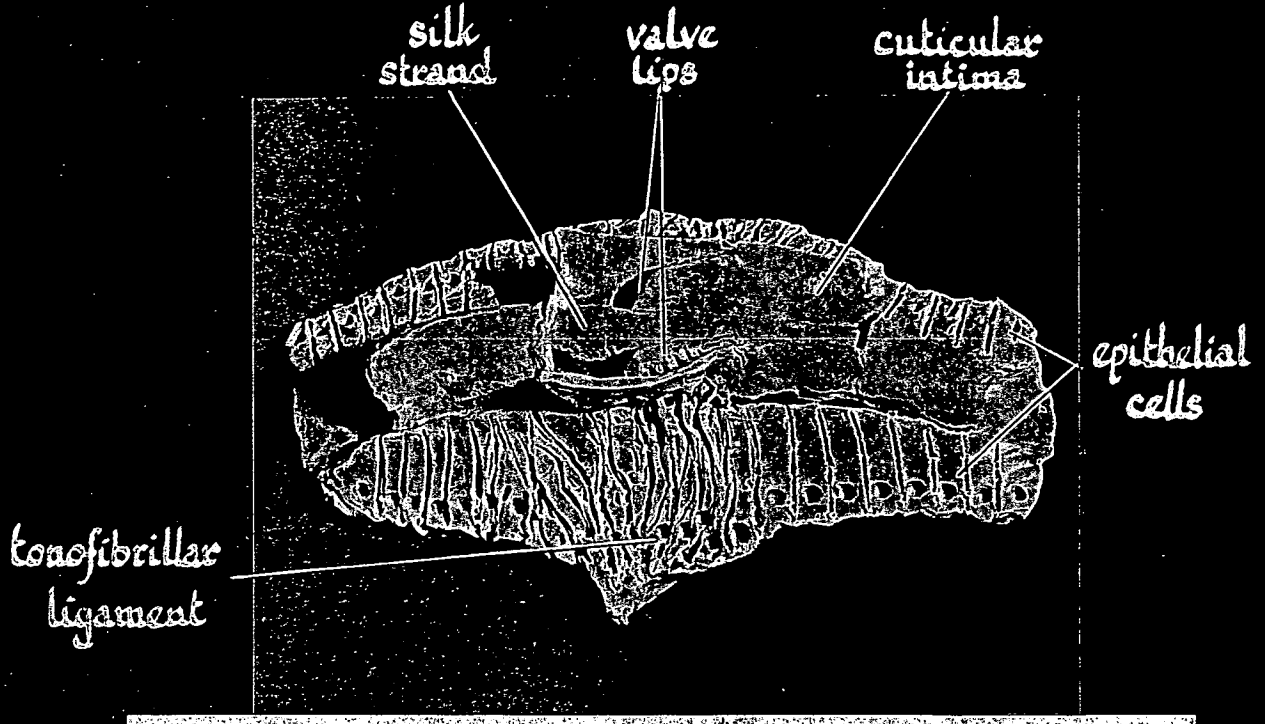

Figure 33. Zygiella, plasticine model of the control valve showing the cuticular intima of the duct slit open and folded back, revealing the two lips lying either side of the silk strand.

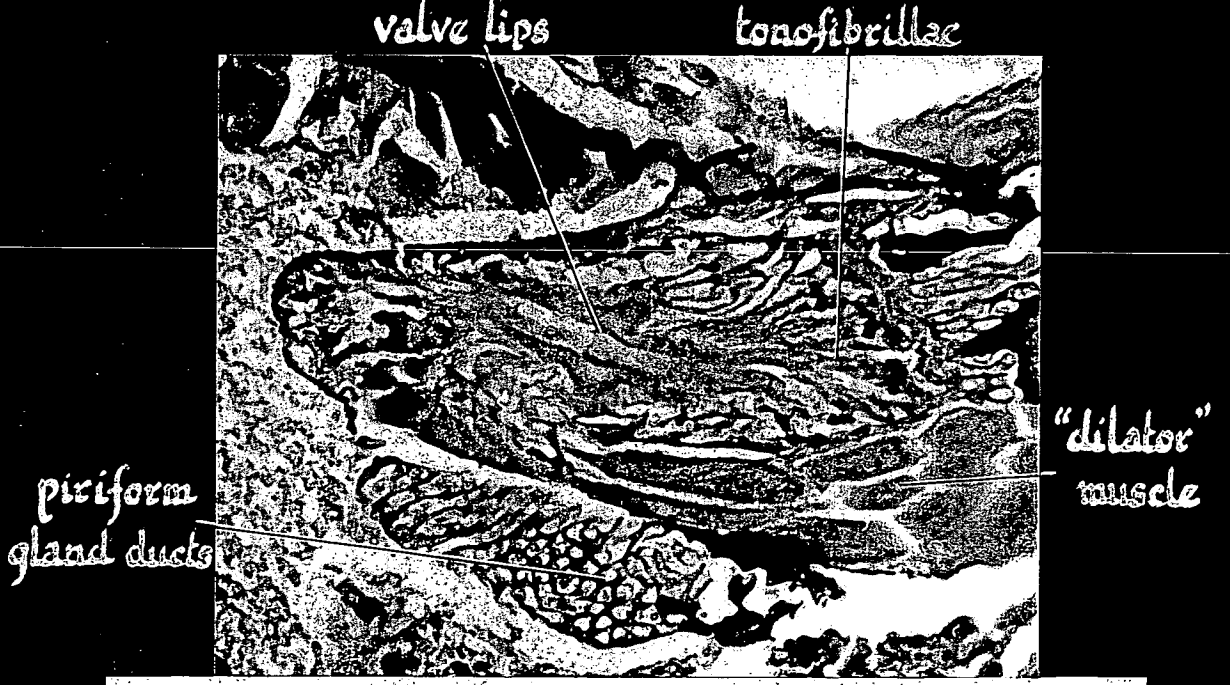

Figure 34. Zygiella, horizontal section through the base of the anterior spinneret, showing the control valve in longitudinal section. (x500 approx.)

that the spinnerets can be moved outwards individually, whereas contraction of the flexors will result in all the spinnerets moving inwards together. Spreading of the spinnerets may also be achieved by internal hydrostatic pressure, as is the case with extension of the legs. The infolded and extended positions of the spinnerets are illustrated in Figure 35.

A detailed study of the internal musculature of the anterior spinneret revealed that the muscles attached to the valve lips are positioned so as to pull them apart, thus allowing the silk to pass more easily down the duct. Closure of the valve must be effected by the inherent elasticity of the duct walls, or by a kinking of the duct, because no muscles are present which could achieve the closure directly.

When the dragline is pulled from an unanaesthetised spider, it can be seen under the microscope that infolding of the spinnerets results in the silk being gripped, whereas the silk can be pulled out easily if the spinnerets are spread out. An examination of Figures 28 and 29 reveal that the spinnerets are spread when the spider is actually dropping on its dragline, but are closed together when it is stationary. It may also be seen that the silk is being gripped by the hind leg in the latter case. It is therefore suggested that closure of the valve may result from a kinking of the duct, associated with the infolding of the spinnerets and contraction of the flexor muscles.

Figure 35. Zygiella, two individuals in ventral view, arranged to illustrate the "infolded" and "spread" positions of the spinnerets. (x6 approx.)

B. The mechanics of silk spinning

In the spider, the dragline silk has to pass down a long, thin duct leading from the body of the gland to the spigot on the anterior spinneret. The duct is looped back on itself, and must present a considerable frictional force opposing the movement of the silk. The loop increases the length of the duct by about two and a half times, and this increase in length probably serves to ensure that the protein molecules of the silk are correctly orientated and arranged so as to form the thread. The greater the degree of orientation, the greater will be the strength of the silk (Savory, 1952), and it is clearly important that the dragline should be as strong as possible.

An orb-web spider, when disturbed, drops very rapidly from the web on its dragline, and then stops for a short period before either continuing its descent, or climbing back to its original position. This initial drop appears each time to be limited to about a foot with the smaller spiders, although a slower descent may be maintained over a much longer distance. Normally, the spider may stop by gripping the dragline with its fourth pair of legs, but when spiders with these legs amputated were tested by making them drop from an artificial support, it was found that they, too, could only produce short lengths of silk at any one time, when falling at a very fast rate.

These observations suggested that there might be a relationship between the speed of silk extraction and the amount of silk available, due to some mechanical property of the spinning apparatus. An investigation was therefore made into the tensions required.

to pull the dragline out at various speeds, in the hope of revealing a relationship between these two factors which might explain the observed behaviour of the spider.

1. Experimental technique

Individuals of Zygiella were anaesthetised with ether until no further movements could be observed, and were then suspended in a small loop of cellotape from a simple torsion balance, which was made from a thin glass tube fixed to a short length of copper wire. The dragline was then picked up with a needle, and given a turn round a paper drum mounted on a cardboard disc, which could be rotated at various speeds by a small electric motor. Figure 36 shows the arrangement of the apparatus.

Readings were made by taking photographs of the whole apparatus, thus obtaining simultaneous records of the tension in the silk and the speed of revolution of the drum. The silk tension was calculated by subtracting the weight of the spider from the reading on the balance, while the speed of revolution was calculated from the length of arc traced out during the exposure of 1/30th of a second by a small flash-light bulb, mounted near the periphery of the blackened cardboard disc which carried the drum.

After an initial photograph to record the weight of the spider, the drum was set revolving at a gradually increasing rate until the silk broke, or the maximum speed of the apparatus was reached. Photographs were taken at intervals throughout this period, and one which was taken during an actual experiment is shown in Figure 37. The balance was calibrated against a standard torsion balance,

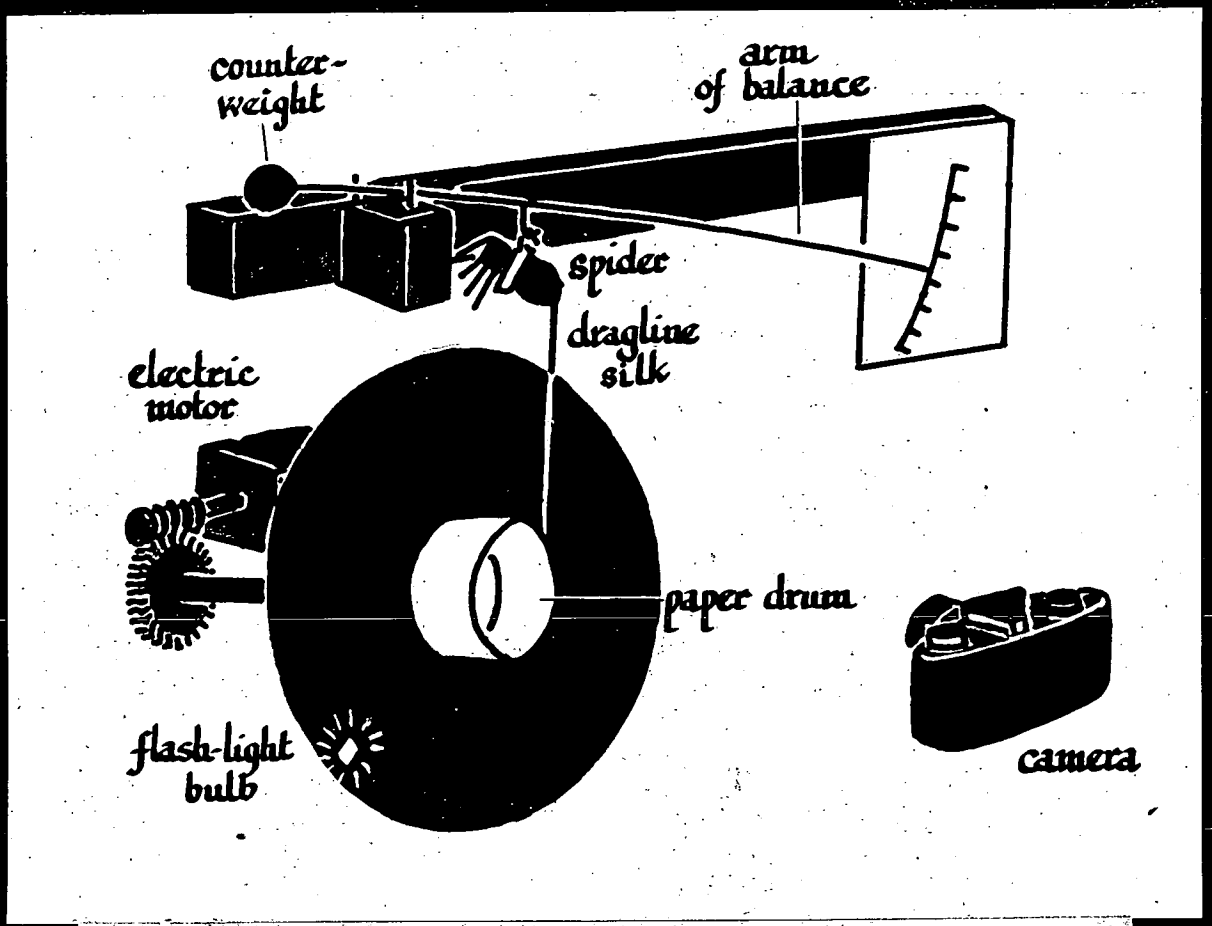

Figure 36. Diagram showing the main features of the apparatus used for extracting dragline silk from spiders at different rates. The spider was suspended from a simple torsion balance, and the dragline silk was wound out on a paper drum, turned by a small electric motor. For further details see text.

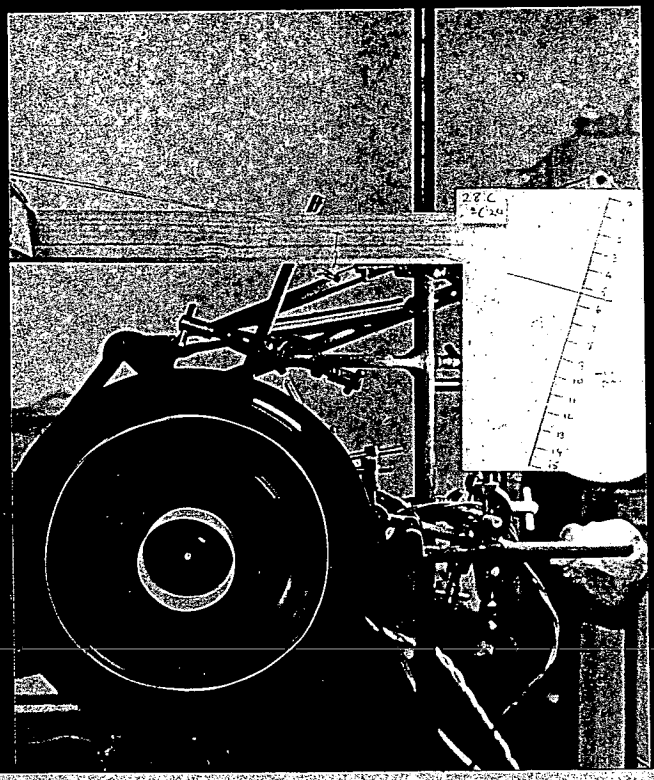

Figure 37. Photograph of the silk extraction apparatus taken during an experiment, showing the arc traced out by the flash-light bulb and the reading on the balance scale, from which the instantaneous values for speed of extraction and tension in the silk were calculated. For further details see Figure 36 and text.

and the spider was weighed on the same balance after each run, thus maintaining a check on the calibration.

2. Results

Graphs were plotted showing speed of extraction/tension in silk (Figure 38), and the readings taken from these graphs are given in Table 1.

The graphs show a linear portion, which may be extrapolated back to the vertical axis to give the "initial tension", followed by a steep and irregular rise in each case, with the exception of Spider 9. Although there appears to be no correlation between the weights of the spiders and either the initial tension or the slope of the linear portions of the various graphs, the speed at which non-linearity intervenes is remarkably constant at about 30 cm./sec., and this has been termed the "critical speed".

It may be added that, when this speed was reached, the balance would show wild fluctuations in tension for little or no increase in speed. Sometimes a sudden, great increase in tension would slow the drum down, whereupon the tension would relax, only to build up again as the drum once more picked up speed. This would seem to indicate that the silk "siezes up" in the duct when the speed of extraction passes a certain critical value.

The results obtained with Spider 9 were exceptional in that there was no measurable initial tension, the slope of the graph was very low, and there appeared to be no critical speed of extraction. It was found, however, that in this case silk from the minor ampullaceal glands had been picked up by mistake, and the results therefore reveal differences between the production of

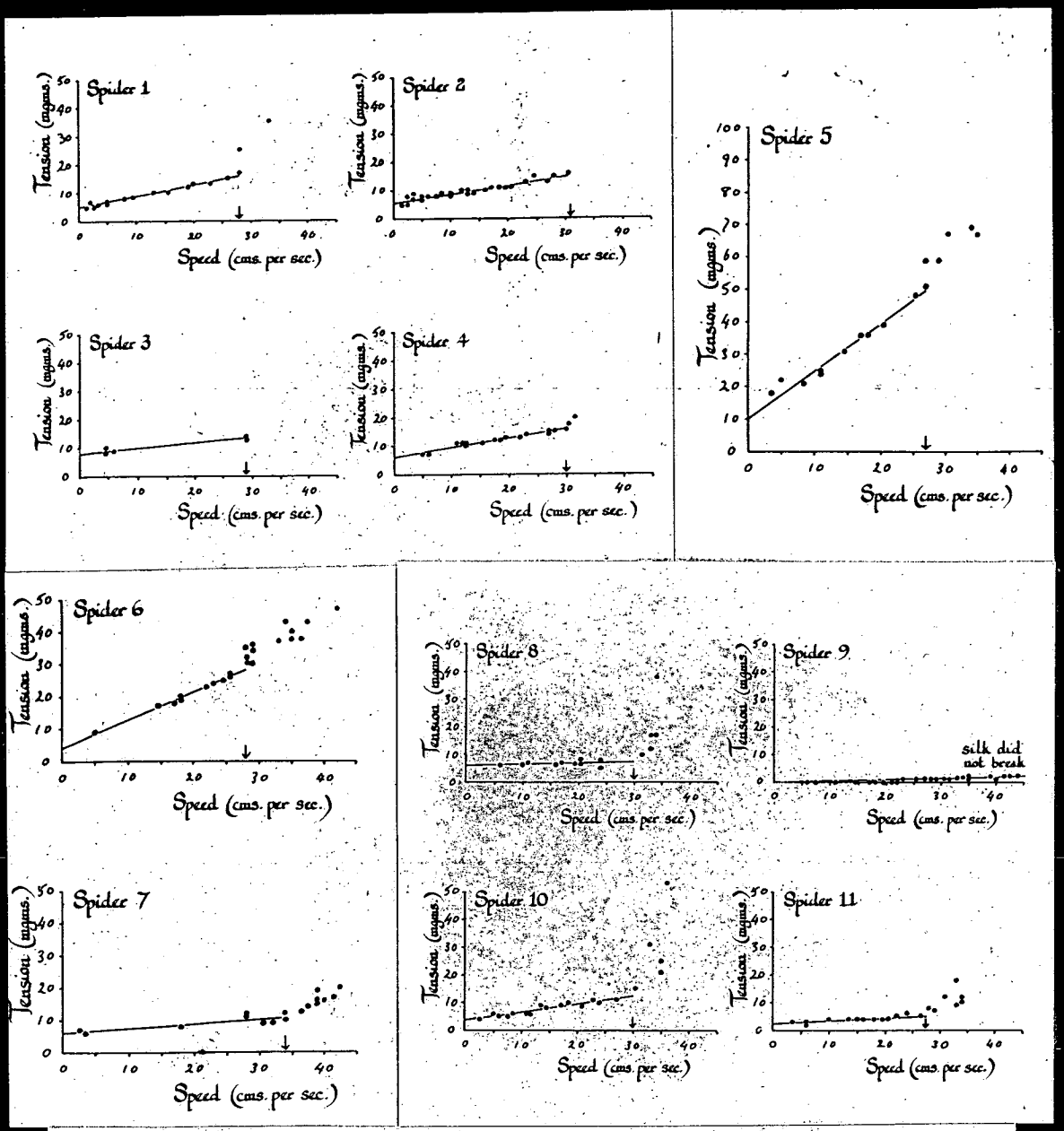

Figure 38. Graphs of tension in silk plotted against speed of extraction for individual adult female Zygiella. The graphs show a linear portion followed by a steep and irregular rise. The speed at which the graph becomes non-linear is termed the "critical speed", and is marked with a small arrow. With the exception of Spider 9, readings were terminated by breakage of the silk. For further details see text.

Serial number	Slope T/S	Critical speed cm./sec.	Initial tension mgms.	Weight mgms.
1	0·419	28	5	24
2	0·317	31	5·5	17
3	0·167	29	8	11
4	0·317	30	6	15
5	1·4	27	10	29
6	0·88	28	4	32
7	0·133	34	6	30
8	0·067	30	6	55
9	0·005	~	0	33
10	0·3	30	3·5	28
11	0·0104	27·5	2·5	55

Table 1. Summary of the results of dragline extraction from Zygiella x-notata (females). The figures for slope, critical speed and initial tension have been taken from the graphs, figure 38. For details see text.

this silk and that from the major ampullaceal glands. These differences are presumably due mainly to the presence of the control valve in the duct of the latter, and may indicate that the initial tension is really a measure of the degree of closure of the control valve.

SILK SPINNING IN SILKWORMS

The silk produced by the silkworm consists of an inner core of fibroin which is covered with a layer of sericin. The sericin is sticky, and enables the silk to adhere to the surface upon which it is spun. The silkworm possesses two identical glands which lie in the body cavity along the side of the gut, and receive tracheal branches from the spiracles in each segment. The glands show no special characteristics of growth and development during the first four instars, but their size increases greatly during the 5th instar, reaching a maximum just at the beginning of spinning. Figure 39 shows dissections of Cynthia larvae at different times during the 5th instar, which illustrate the growth of the silk glands. When mature, the glands can be seen to consist of three sections: a terminal section, the cells of which secrete the fibroin; a central section, which secretes the sericin and in which the silk is stored prior to spinning; and the duct, which leads from the central section to the spinneret.

The ducts from the two glands join before reaching the single centrally placed spinneret, and there is an organ lying between this junction and the spinneret which is known as the "silk press". In structure the silk press is a short chitinous tube, T-shaped in cross-section, through which the silk threads must pass. Muscles run from the walls of the press to the U-shaped prementum, and serve to open the press when they contract. Closure is not effected by muscular action, but by the elasticity of the press itself. Figures 40, 41 and 42 illustrate some of these features in Bombyx.

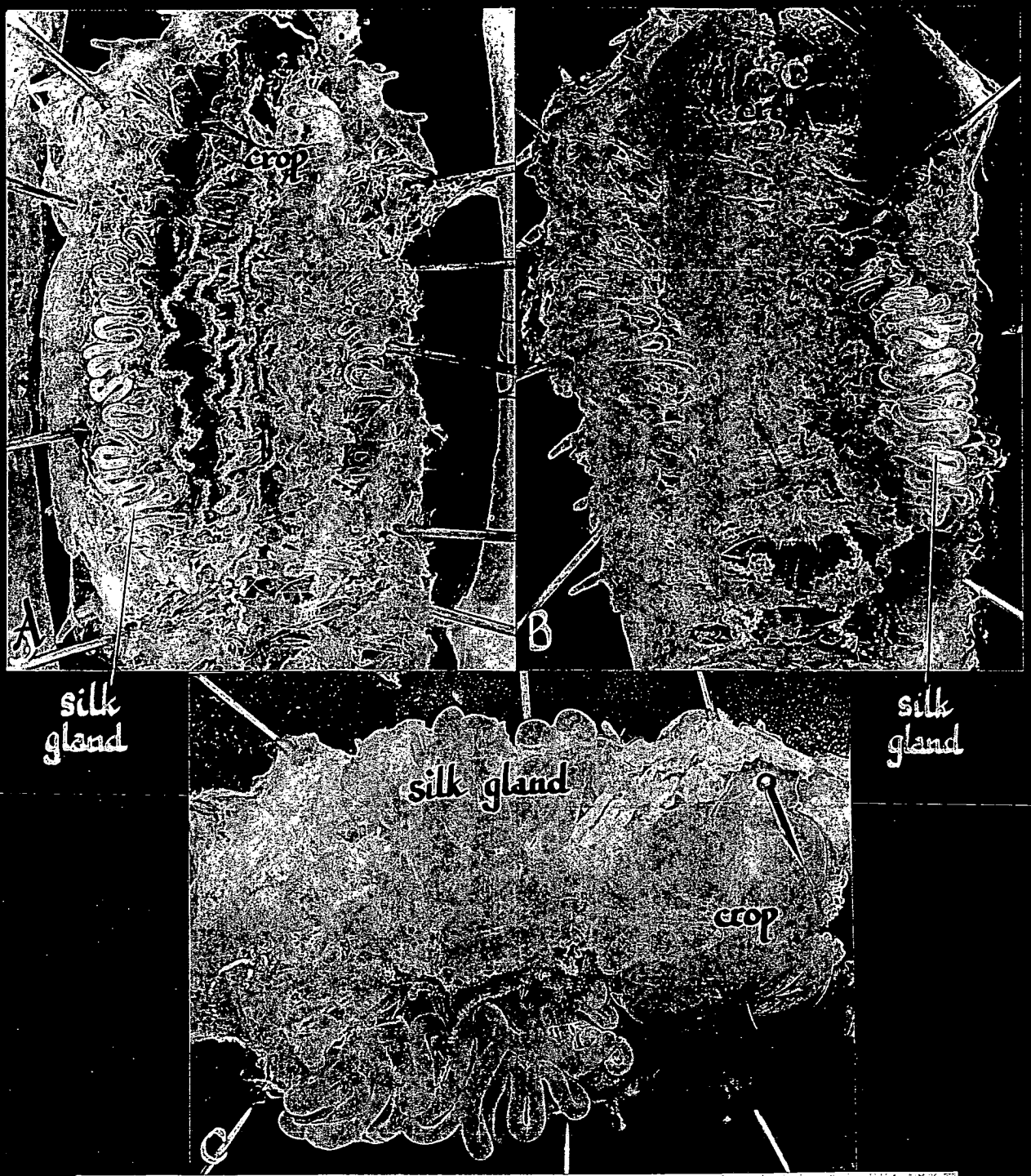

Figure 39. Cynthia, dissected A at the beginning, B in the middle, and C at the end of the 5th. instar, illustrating the growth of the silk glands. (x2 approx.)

Figure 40. Bombyx, horizontal section through the silk press, showing the junction between the two silkgland ducts. (x16 approx.)

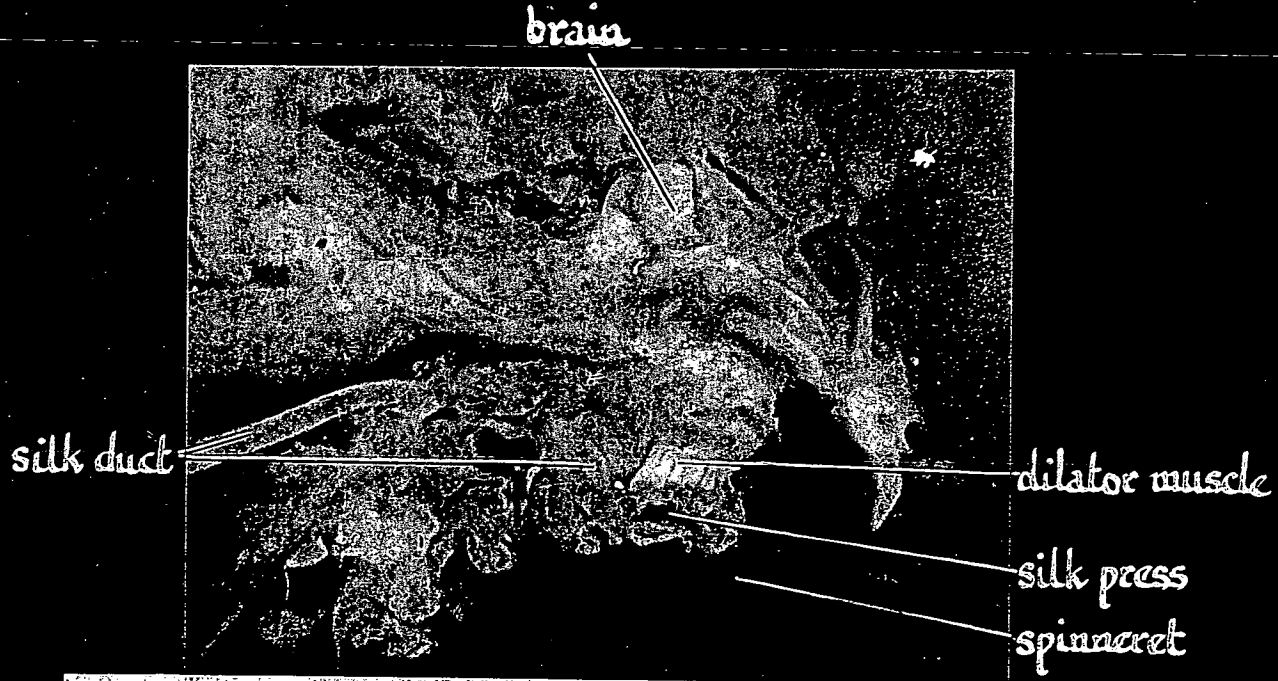

Figure 41. Bombyx, vertical longitudinal section through the head and silk press, showing its position with respect to the spinneret. (x16 approx.)

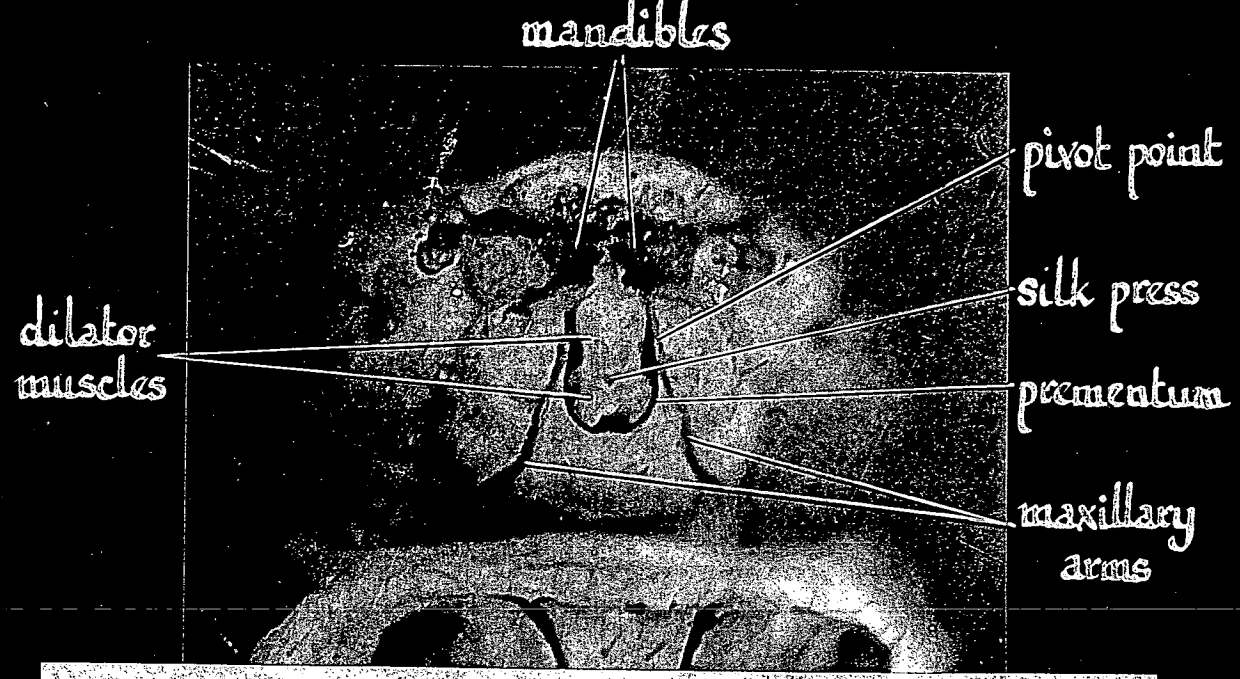

Figure 42. Bombyx, transverse section through the silk press, showing its position in the prementum. The pivot points between the prementum and the maxillary arms are also illustrated. (x16 approx.)

After passing through the press, the two silk strands emerge from the spinneret as a double thread. As mentioned in the previous section, the thread formation depends on the alignment of the protein molecules under tension as the silk is pulled out, and this process depends on the silk being attached to some solid object before the thread can be spun. In order to attach the silk, the spinneret is swung forwards by a set of muscles inserted on the spinning apparatus, pivoting at the point where the prementum rests on a pair of maxillary arms. When in this position, the spinneret has been termed "protracted", and is set so that its tip comes into contact with the surface on which the larva is spinning.

If a spinning silkworm is removed from its cocoon, and the thread is grasped and pulled out, it is found that only a short length can be extracted before the silk "siezes up" somewhere inside the spinneret. Further pulling only results in the silk breaking off at the spinneret. Similarly, it is difficult to extract more than a short length of silk from a spinning silkworm which has been anaesthetised with carbon dioxide. These experiments strongly suggest that the silk is gripped in the press, when the muscles attached to it are relaxed either naturally, or under the influence of an anaesthetic.

Since the silk gland must contain silk throughout the life of the larva, although it only becomes really full towards the end of the last instar, it would be interesting to know how it is prevented from coming out of the spinneret. The peristaltic crawling movements of the larva must tend to force the silk up the ducts, and one can only surmise that it is held back at the press, which

is kept closed until the appointed time for spinning. In Cynthia , the colour of the spinneret darkens over the twenty-four hours immediately preceding spinning, and may indicate a hardening of the spinning apparatus, since cuticular darkening is frequently associated with the hardening process. As this phenomenon, which has been termed the "black tip" stage, occurs before the spinning of the moulting pad, as well as before cocoon spinning, it would appear to be an integral part of the changes associated with moulting and metamorphosis (Figures 43 and 44). It may be that the ability to produce silk depends in some way on the rigidity of the spinning apparatus, and that this is only achieved just before spinning normally takes place. On the other hand, the darkening of the spinneret may be merely incidental, and have no bearing at all on the production of silk. It is interesting, however, that such a colour change takes place, because it indicates that a biochemical change has occurred in the associated cells.

Figure 43. Cynthia, heads of immature and mature 5th. instar larvae, showing the black spinneret of the latter. The spinneret darkens twenty-four hours before the onset of spinning. (x7 approx.)

Figure 44. Antheraea, spinneret of newly moulted larva compared with that of its exuvium, showing the black spinneret of the latter. The spinneret darkens just before the spinning of the moulting pad. (x20 approx.)

RESPIRATORY METABOLISM AND SPINNING BEHAVIOUR IN SILKWORMS

A great deal of attention has been paid to measuring rates of oxygen consumption in silkworms, in relation to the functioning of the cytochrome-cytochrome oxidase system (Schneiderman and Williams, 1953, 1954). However, there have been no serious attempts to correlate changes in oxygen consumption with the spinning phases at each moult and at the beginning of metamorphosis. Neither have there been any detailed studies on the changes brought about in the silkworm's metabolism by starvation, glandectomy and the use of respiratory inhibitors. Efforts were next made, therefore, to investigate the relationship between spinning behaviour and particular levels of metabolism, both under normal and artificially induced conditions.

A. The relation of respiratory rate to spinning periods

Hsueh and Tang (1944) have shown that a lowering of the respiratory rate occurs at each moult, and Schneiderman and Williams (1953) have indicated that there is a precipitous fall in the respiratory rate during the onset of metamorphosis. No detailed information, however, was given by these authors about the different levels of respiratory rate in relation to the spinning periods, and so attention was next directed towards this relationship.

1. Materials and Methods

Oxygen consumption measurements were performed on Cynthia, Antheraea and Telea larvae during the beginning of metamorphosis, and also during the moulting period from the 3rd to the 4th instar.

The oxygen consumption was measured manometrically using the standard Warburg technique, the silkworms being placed in vessels of approximately 42 cc. capacity. In the case of the Telea larvae, larger vessels had to be used, of approximately 100 cc. capacity. The carbon dioxide was absorbed by means of a loose roll of filter paper moistened with 1/2 cc. of N/10 potassium hydroxide solution, placed at the bottom of the vessel. The moistened roll was covered with either more filter paper, or with a layer of non-absorbent cotton wool, so as to prevent the larvae from coming into contact with the alkali.

Measurements were made daily, readings being taken every five minutes over a period of half an hour, or until they had remained approximately constant for at least a set of three. An average figure was calculated from these, and was taken as the measure of the oxygen consumption.

The pupal weight was used to calculate the rate of oxygen consumption over the period of the onset of metamorphosis, following the procedure of Schneiderman and Williams (1953) to obviate the difficulties arising from the changing weight of the larva. The rate of oxygen consumption over the moult was calculated using the weight of the newly-moulted larvae.

2. Changes in respiration during normal larval moulting

Figure 45 shows the changes in oxygen consumption of Antheraea larvae over a period of the moult from the 3rd to the 4th instar. The curve represents the average taken from six larvae. It is evident that the lowest rate occurs at the time of the moult, and

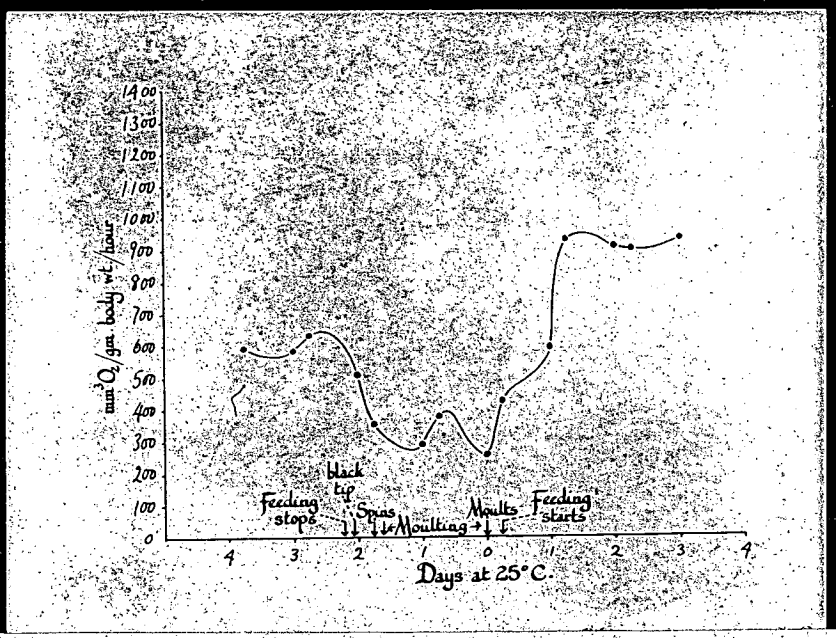

Figure 45. Graph showing the average changes in rate of oxygen consumption of normal Antheraea larvae over the period of the moult from 3rd. to 4th. instar. It can be seen that spinning occurs after the rate has fallen to a low level, that the lowest rate coincides with moulting, and that feeding is resumed only after the rate has shown a significant rise.

that there is both a very rapid drop just before the onset of the moult, and a rapid rise at the end of it. The "moulting" period shown in Figure 45 is characterised by the retraction of the underlying tissues from the head capsule, which gives the larva a typical "head-down" posture.

Feeding stops just at the beginning of the fall in oxygen consumption, and the moulting pad is spun after the rate has reached a low level. The spinning of this structure takes only about an hour.

The resumption of feeding occurs about 6 hours after completion of the moult but the rate of oxygen consumption has already risen significantly by this time. It is interesting to note, however, that normal faeces are passed by the larva before feeding begins, which indicates that the gut resumes its normal digestive processes previous to any fresh food being ingested. It was shown by dissection, however, that the gut retains some green, undigested leaf fragments over the period of the moult, so that the gut has something to work on when it resumes normal functioning.

3. Changes in respiration during the onset of normal metamorphosis

The results shown in Figure 46 illustrate the changes in oxygen consumption occurring at the onset of metamorphosis in Cynthia. The maximum rate of 1400 mm^3O_2/hour occurs during the feeding stage, about two and a half days before spinning. Thereafter the rate drops sharply, reaching about half the maximum rate at the onset of spinning, namely 740 mm^3O_2/hour. It continues to fall throughout the two-day spinning period, reaching a level of 250 mm^3O_2/hour

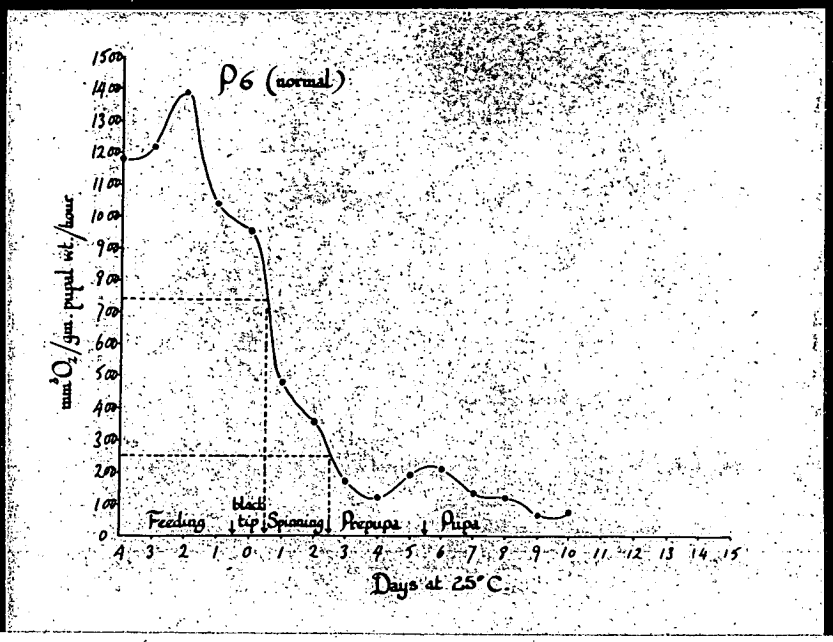

Figure 46. Graph showing the changes in rate of oxygen consumption of a normal Cynthia larva over the period of the onset of metamorphosis. The maximum rate occurs about two and a half days before spinning, and thereafter falls rapidly. It can be seen that spinning occurs over a two-day period during which the rate continues to fall, and that there is a small rise in the rate prior to pupation.

as the larva enters the three-day prepupal stage. A small rise in the rate occurs just before pupation, but after this the rate again falls to the very low levels characteristic of the pupal stage.

The first overt sign of impending metamorphosis is the cessation of feeding. Coincident with this, the tip of the spinneret darkens, and the gut is completely emptied, the last food eaten passing out green and undigested. This "black-tip" stage occurs 24 hours before spinning begins, and is therefore associated with the suspension of gut function, and provides a convenient marker for timing the experiments.

It can be seen, therefore, that the decline in metabolism begins before the onset of spinning, and that spinning occurs over a two-day period, during which the oxygen consumption is at a low and still declining rate.

B. The effect of starvation on respiration and behaviour of feeding silkworms

Since it appeared that the changes in respiratory rate might be coupled to changes in the metabolism of the gut, the effect of starvation on the oxygen consumption of feeding larvae was next investigated.

Accordingly, a number of 4th instar Antheraea larvae were removed from their food-plant, and it was found that their rates of oxygen consumption showed an exceptionally rapid fall. Figure 47 shows that the rate was approximately halved after the first six hours starvation at 25°C.; that the decline continued for a further six hours, only at a slower rate; and that the oxygen consumption subsequently showed a slight recovery. When the larvae were again

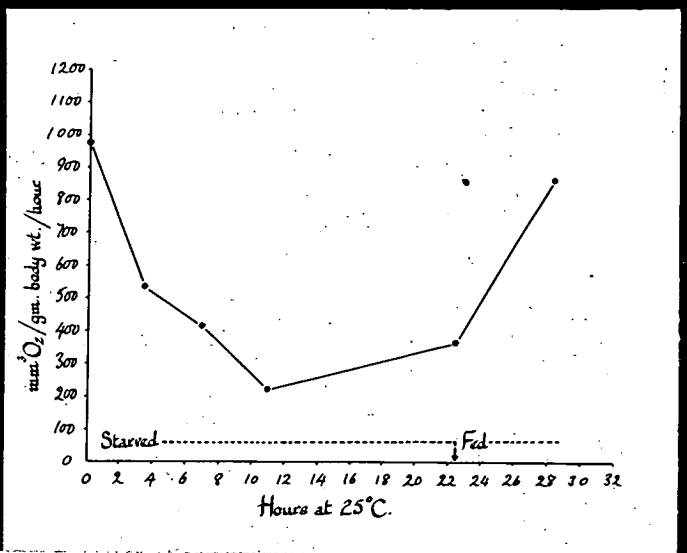

Figure 47. Graph showing the average changes in rate of oxygen consumption of Antheraea larvae just removed from their food plant. It can be seen that there is a spectacular fall in the rate which levels out after about eleven hours at 25°C., and that a spectacular rise in the rate occurs after the larvae were again allowed to feed.

presented with food and allowed to eat, the rate showed a very sharp rise, almost equivalent to the fall recorded at the beginning of the experiment.

At the point of lowest oxygen consumption, the larvae showed a striking failure of locomotory co-ordination between the front and rear parts of the body. The abdomen appeared to be semi-paralysed, and the whole larva showed spasmodic twitching. When offered fresh leaves, some of the larvae were able to feed, and the food was again removed as soon as this was observed. Of the other larvae, some remained in the semi-paralysed condition and did not react to the proffered food, while the rest were the most affected of all, and showed only small side to side movements of the head. It is interesting to note that one of the latter larvae not only had the lowest oxygen consumption, but also that its head movements were accompanied by protraction of the spinneret.

After a further five hours, all the larvae had recovered normal locomotion, their rates of oxygen consumption had risen slightly, and they were all able to feed when again offered fresh food.

It seems then, that the overall respiratory rate of the silkworm is greatly influenced by gut metabolism, and even by the presence or absence of food in the gut. It may be significant that the decline in the rate of oxygen consumption caused by the withholding of food, is more than sufficient to cover the declines observable both at the moult and at metamorphosis.

C. The relation of glandectomy to respiratory rate and spinning behaviour

Van der Kloot and Williams (1953b) reported that removal of

the silk glands from last instar Cecropia larvae had drastic effects on the cocoon-spinning behaviour. When the operation was performed early in the last instar, the larvae made very few movements at the time of spinning, while continuous figure-of-eight movements were shown throughout the spinning period when the operation was performed just at the onset of spinning. If the spinnerets were blocked so that no silk could be produced, normal spinning movements occured at the usual time, and when one gland was removed and the other severed at some point along its length, the larva went through the motions of spinning a complete cocoon, although it ran out of silk during the process. These results show that the presence of the silk glands themselves is a prerequisite for the performance of normal spinning movements. The figure-of-eight movements shown by the completely glandectomised larvae were interpreted by Van der Kloot and Williams as concerned with visual orientation, and not with spinning.

Similar experiments carried out by Akao (1943) showed, however, that glandectomy led to the accumulation of aminoacids in the blood, which could reach a toxic level at about the time of metamorphosis. He reported that the percentage of larvae dying as a result of this "aminoacidaemie" could be lowered by starving the larvae towards the end of the instar. Umeya (1926) states that, when the spinneret is removed so as to prevent the spinning of silk, the glands continue to increase in size at the expense of the other tissues, and may finally rupture. The animal frequently dies from the effects of this operation.

It is likely that glandectomy affects spinning behaviour through the accumulation of aminoacids and their toxic action, rather than

through any more direct effect, although the brain may also be dependent on proprioceptive input from the spinning apparatus. There appears to be little evidence that the silk glands function as endocrine organs themselves. It was considered necessary, however, to repeat the experiments of Van der Kloot and Williams, with a view to obtaining more precise information regarding the relationship between the silk glands and the spinning behaviour.

1. Materials and Methods

Cynthia larvae which had recently entered the 5th instar were chosen for the bulk of these experiments, although some were operated on towards the end of the 4th instar, and some just at the beginning of spinning.

The larva to be operated on was placed on a block of plasticine in a well directly underneath a binocular microscope, which was fitted with a foot focussing control. A tube led from a commercial cylinder of carbon dioxide directly into the well, and, by adjusting the gas flow, the larva could be kept continuously anaesthetised. The use of carbon dioxide as an anaesthetic for insects has been discussed by Williams (1946). A polythene sheet was adapted to cover that part of the bench immediately round the well, and the whole area was regularly swabbed with 96% alcohol. This arrangement, together with the boiling of instruments and the surface sterilisation of the larva, served to maintain sterile conditions throughout the operation, and were considered worthwhile, although it is known that insects are particularly immune to bacterial infection (Wigglesworth, 1950). Figures 48 and 49 show the operating table, with the larva in position.

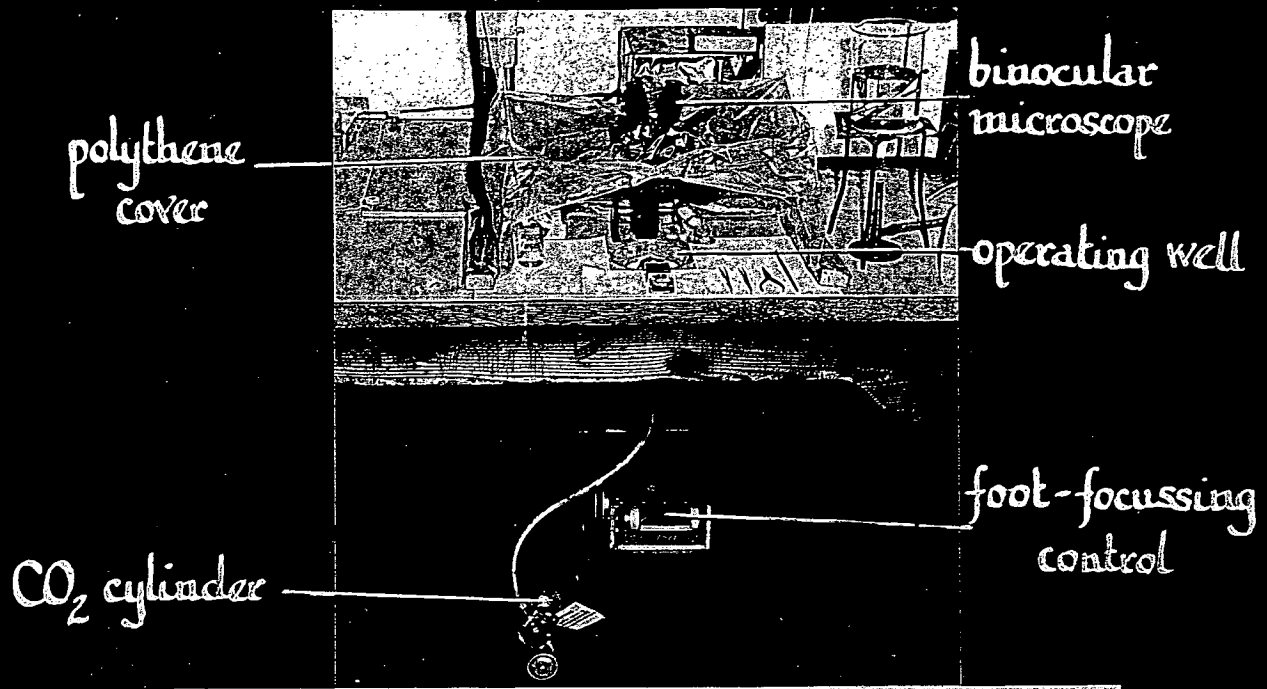

Figure 48. Photograph of the apparatus used during operations on the silkglands of Cynthia larvae. For details see text.

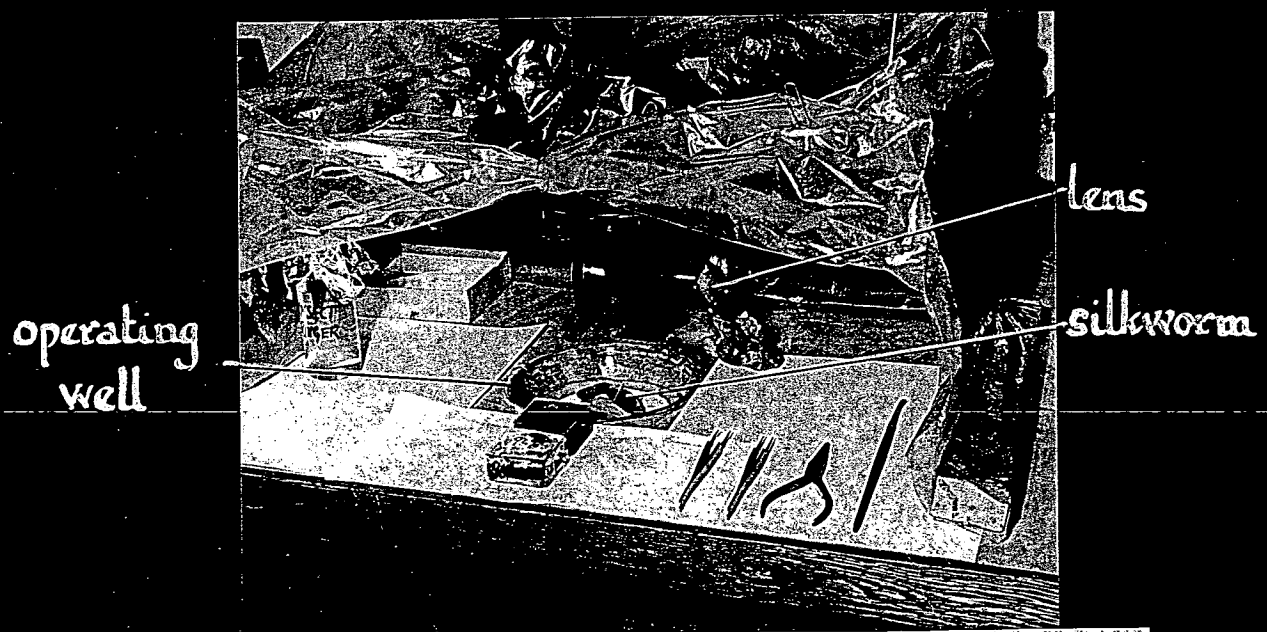

Figure 49. Close-up photograph of the apparatus used during operations on the silkglands of Cynthia larvae, showing a larva in position on the operating block.

After the larva had relaxed under the anaesthetic, it was placed on its side on the operating block and was fixed in position with crossed pins. It was swabbed locally with 96% alcohol and dried, and an incision was made in the region of the 4th abdominal spiracle with the aid of two pairs of watchmaker's forceps. The silk gland was located, and was carefully extracted by pulling it out in short lengths at a time, severing the tracheal connections as they appeared. The silk glands in Cynthia were found to be soft and easily broken, especially near the tip of the secretory portion, and great care was needed to extract them whole. When one had been removed, it was placed in a watchglass and examined to see if it was complete (Figure 50). The incision was then closed with an aluminium clip, and moistened with 96% alcohol to coagulate any exuded haemolymph.

When the operation had been completed for one side, the silkworm was turned over and the process repeated for the other side. The larva was then placed in a refrigerator at 5°C. for 48 hours, in order to offset the effects of surgical shock (Van der Kbot and Williams, 1954).

The oxygen consumption of the successfully operated larvae was followed until pupation or prior death, while unoperated larvae, and some in which the glands had been only partially removed, acted as controls. Kymograph tracings were taken during the "spinning period", which was identified by its relation to the cessation of feeding and the darkening of the spinneret, by the method outlined by Van der Kloot and Williams (1953b), and the movements of both normal and glandectomised larvae were compared by means of a short motion film.

Figure 50. Cynthia, silkgland after removal from the body cavity, showing the ends of the tracheal branches severed during its extraction. The duct and the tip of the secretory portion of the gland are also illustrated. (x5 approx.)

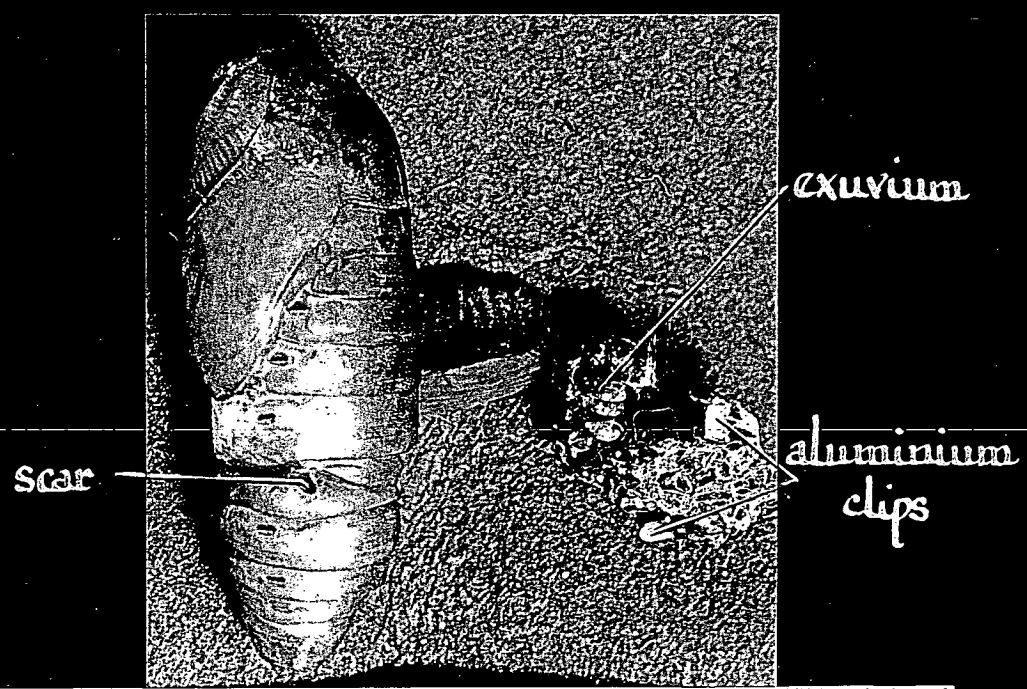

Figure 51. Cynthia, normal pupa and exuvium formed by a glandectomised larva. The aluminium clips used to close the incisions are attached to the exuvium, while the scar left by the operation can be seen on the side of the pupa. (x3 approx.)

2. Effects of removing silk glands on respiratory rate

The graph shown in Figure 52 gives the changes in oxygen consumption for Cynthia larvae which had their silk glands completely removed early in the 5th instar. The graph is an average of six larvae, and is valid over the "spinning period" in spite of many premature deaths.

The rate of oxygen consumption of the glandectomised larvae was maximum about two and a half days before the "spinning period", reaching 1200 mm^3O_2/hour, and at the beginning of the "spinning period" it had fallen to 510 mm^3O_2/hour. These figures are below those of 1400 mm^3O_2/hour and 740 mm^3O_2/hour for normal larvae at the same periods, and may reflect an overall lowering of the metabolism, or indicate that the silk glands are of considerable importance in maintaining the normal level of oxygen consumption at this stage.

The dying of the glandectomised larvae before completing metamorphosis may be attributed to "aminoacidaemie"(Akao, 1943), but it is clear that this does not always prove fatal, since one larva survived to pupate normally and emerge as a normal adult.

Figure 51 shows the pupa with its exuvium, and illustrates the damage done by the operation. The epidermis heals under the little aluminium clips used to close the incisions, and these clips are sloughed off with the old larval skin at ecdysis.

Some larvae were operated on towards the end of the 4th instar, in order to study whether the removal of the glands at this stage would upset the normal moulting process. Since the glands only start to grow rapidly in the 5th instar, the danger that "aminoacidaemie" might affect the result was avoided. It was found that the

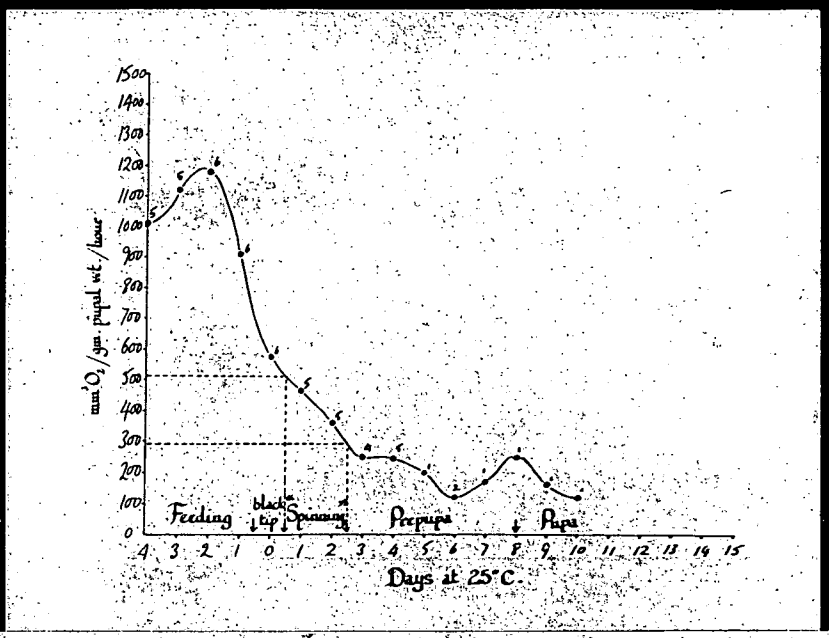

Figure 52. Graph showing the average changes in rate of oxygen consumption of glandectomised Cynthia larvae over the period of the onset of metamorphosis. It can be seen that the rates are lower than those of normal larvae over the same period. The small figures beside the points denote the number of larvae whose rates were averaged to obtain the plotted values.

operation had no observable effect on the process of normal larval moulting, and indicated that moulting did not depend in any way on the presence of the silk glands at this stage.

3. The effects of removing silk glands on spinning movements

In studying the movements performed by larvae during the spinning period, it was observed that individuals operated on just at the beginning of spinning showed almost continuous figure-of-eight movements throughout this time. Those larvae, however, which had their glands removed early in the instar, moved very seldom during the spinning period, although their feeding behaviour had been normal. Some figure-of-eight movements were performed by the latter larvae, mostly after receiving a generalised stimulus, such as being tipped out of their vials onto the bench. It is significant, however, that only movements shown by any of the glandectomised larvae were figure-of-eight movements, and that close examination of the position of the spinneret revealed that it was protracted.

Kymograph recordings taken during the spinning period (Figure 53) showed that the normal larvae performed both small continuous movements, and gross changes of body position, during spinning (the "turning around" of Van der Kloot and Williams, 1953b), and that the movments of larvae which had the tips of the secretory portions of the glands left in the body cavity were essentially similar, only smaller in amplitude. The larvae from which the glands had been removed, however, showed only very small, continuous movements, in no way comparable to those shown by larvae just removed from the food plant. The latter showed an initial burst of activity for about 20 minutes and then remained quiet. These results were confirmed by

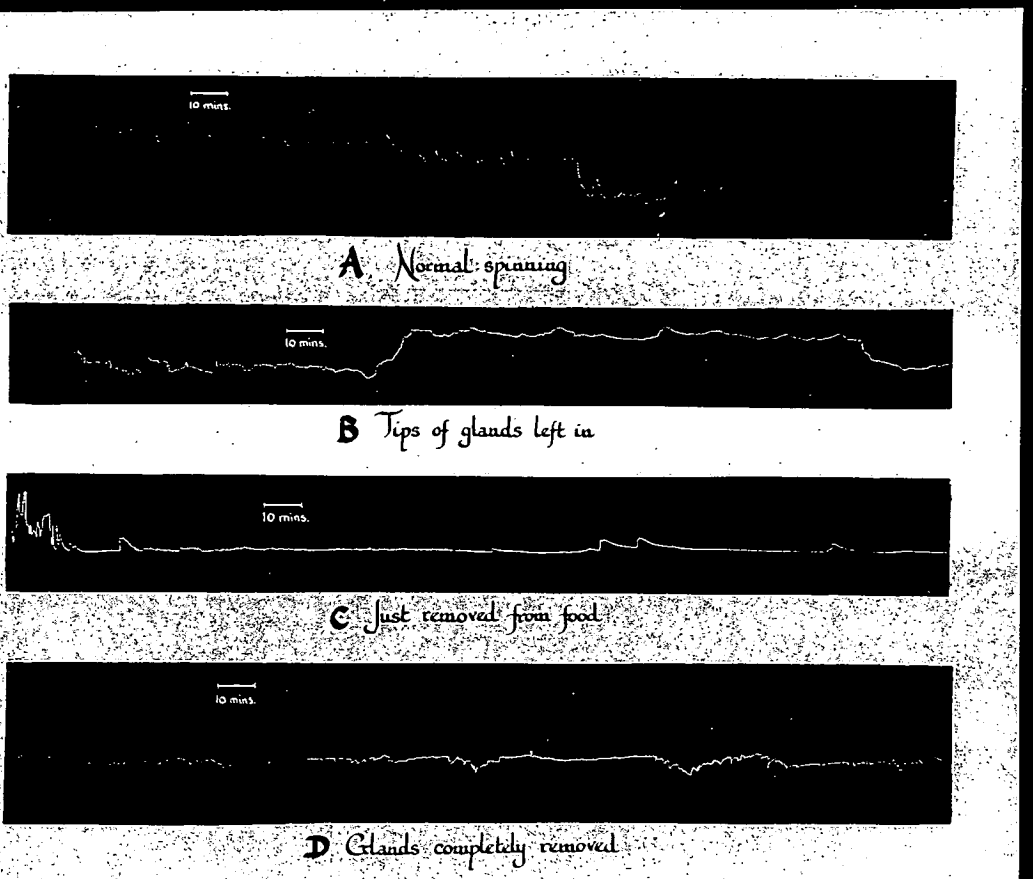

Figure 53. Portions of kymograph recordings illustrating the cocoon spinning movements of a normal Cynthia larva (A), compared with the "spinning" movements of larvae which had either all except the tips of the silkglands removed (B), or had the silkglands completely removed (D). Also shown is the recording made by a normal feeding larva just removed from the food plant. It can be seen that B resembles A closely, whereas D lacks the large body movements shown in A and B. C bears little resemblance to any of the others.

critical examination of the motion film.

In normal larvae, spinning movements are characterised by the continuous protraction of the spinneret, whereas "searching" movements, as shown by larvae removed from their food, are characterised by the use of the labial palps to investigate the surface on which they are walking. In view of this, it is concluded that the figure-of-eight movements shown by glandectomised larvae are true spinning movements, and that spinning behaviour is not completely suppressed by the removal of the silk gland, although it appears to be greatly modified.

D. The suppression of respiratory metabolism and its effect on spinning behaviour

In the course of some preliminary investigations it was found that phenylthiourea, a copper-complex inhibitor, markedly reduced the oxygen consumption of feeding Cynthia larvae when injected into the body cavity. This treatment also affected the nature of the cocoon spun later by the silkworm, and, in some cases, induced the formation of pupae showing a number of larval characteristics.

It therefore appeared that, by exploiting the anti-metabolic action of phenylthiourea, some insight might be gained into the relationship between spinning behaviour and general metabolism. Various amounts of the compound were therefore injected into larvae just before the spinning period, and the effect on both the cocoon-spinning and the oxygen consumption was studied. The metabolic effect of the compound was also followed in feeding larvae, in order to avoid the complications arising at metamorphosis.

Since phenylthiourea is known to block the action of blood phenolase in insects (Dubois and Erway, 1946) and has been used to prevent the formation of toxic phenolic substances after surgical operation (Williams, 1952), it was also considered to be of interest to determine whether a relationship existed in the silkworm between the blocking of blood phenolase activity and the depression of respiration.

1. Use of phenylthiourea to depress respiratory metabolism.

Individuals of 5th instar Cynthia larvae were anaesthetised with carbon dioxide, and crystals of phenylthiourea were inserted into the body cavity through incisions made in the abdominal wall. The incision was then closed with an aluminium clip, and the exuded haemolymph coagulated with a small quantity of 96% alcohol. The crystals appeared to dissolve readily in the haemolymph, in contrast to their behaviour in pure water, so that they had to be inserted quickly, and deposited as far from the opening as possible, in order to minimise any loss of the compound through bleeding.

The larvae were treated with either 0.5, 0.75, 1.0 or 1.5 mgrams of phenylthiourea, and weighed between 3 and 4 gms each. They invariably made a rapid recovery from the effects of the anaesthetic, and the changes in rate of oxygen consumption subsequent on the operation were followed by the standard Warburg technique, as previously described.

Figure 54 shows the depressant action of the various amounts of inhibitor on the rate of oxygen consumption. When 0.5 mgms. were inserted into the body cavity, the oxygen consumption showed a significant decrease in rate. Subsequently, the rate increased

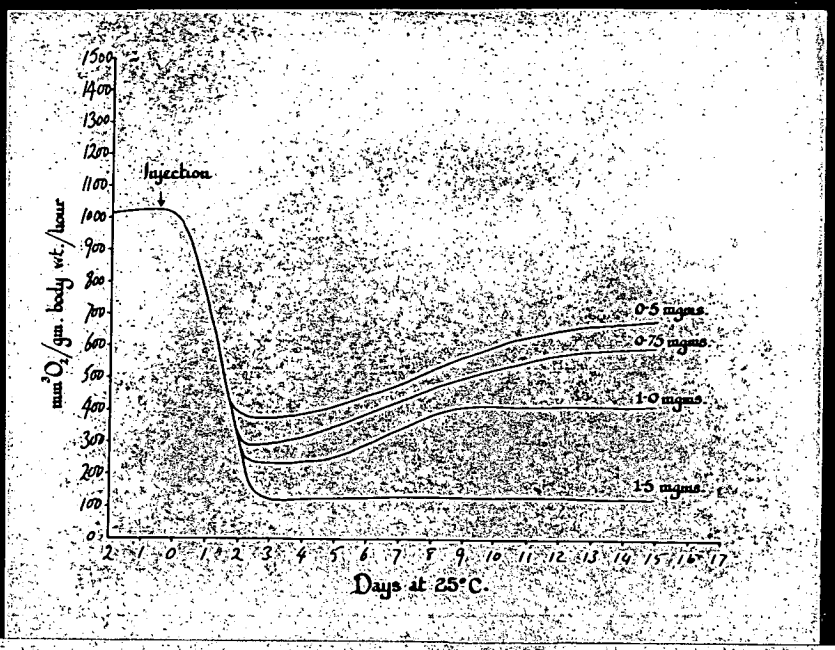

Figure 54. Graphs showing the changes in rate of oxygen consumption of feeding 5th. instar *Cynthia* larvae after treatment with various amounts of phenylthiourea. The curves show that the depressant effect of the compound is proportional to the amount inserted into the body cavity. For further details see text.

without, however, regaining the pre-treatment level. At the onset of spinning the rate was about 650 mm^3O_2/hour.

Treatment with 0.75 mgm. had a depressant effect that was more marked. The initial decrease in rate was again followed by a rise, but this, however, failed to reach a level above 500 mm^3O_2/hour before spinning was initiated. Moreover, the larvae treated with this amount were rendered incapable of constructing normal cocoons, although they were not prevented from pupating.

A striking fall in the rate occurred after treatment with 1.0 mgm. of the inhibitor, and the larvae generally became immobilized after a delay of several hours. This induced quiescent state usually lasted about two days, and was followed by a gradual recovery of movement, which was accompanied by an increase in the rate of oxygen consumption. The rate, however, seldom reached a level above 400 mm^3O_2/hour before spinning took place. Though the usual two-day period was spent in spinning by these larvae, it was observed that they failed to produce more than a limited sheet of silk.

Amounts of phenylthiourea in excess of 1.5 mgm. were usually lethal to the silkworm used. They invariably brought about a precipitous fall in the respiratory rate, and so completely immobilised the treated silkworm, that they seldom showed any signs of recovery.

These studies indicated that the depressant action of phenylthiourea on the rate of oxygen consumption in Cynthia is proportional to its concentration in the blood and tissues. Moreover, it will be apparent that this silkworm seems capable of reversing the depressant action of the inhibitor on its respiratory metabolism, provided the amount administered is less than the lethal dose.

2. Effect of phenylthiourea on blood phenolase in feeding stages

Efforts were next made to determine the inhibitory effect of phenylthiourea on the blood phenolase. Though it was unlikely that the site of the depressant action on the respiratory rate was the blood phenolase, it was nevertheless desirable to estimate the ability of this compound to inactivate a copper-catalyst which was known to be present in the blood and probably also in the tissues.

Phenolase (tyrosinase) is distinguished from other copper enzymes by its ability to catalyse the insertion of an hydroxyl group into monohydric phenols, and the oxidation of the resulting o-diphenols to their corresponding o-quinones (Dawson and Tarpley, 1951).

It is generally accepted that the enzyme is present in the blood of insects, and that, when the blood is exposed to air, it turns brown, owing to the enzymatic oxidation of polyphenols to form melanin-like substances. The addition of a diphenol substrate, such as dihydroxyphenylalanine, to the blood of an insect, results in the rapid formation of melanin due to the reaction being catalysed by the phenolase. Melanin is not deposited if phenylthiourea is also added, and the inference to be drawn is that the inhibitor suppresses the development of melanin, in blocking the activity of the phenolase.

The following tests were carried out to permit an estimation of the extent to which the blood phenolase of Cynthia could be inhibited by the introduction of phenylthiourea. Silkworms weighing about 3 to 4 grams were treated with amounts of either 0.45 mgm. or 1.0 mgm. of the compound by inserting the crystals into the body cavity, as

already described. Samples of blood were taken from these silkworms twenty-four hours later. A solution of dihydroxyphenylalanine was added added to the samples, so that 1 cc. of the final solution contained 1 mgm. of the phenol substrate. Figure 55 illustrates samples of blood from silkworms previously treated with the inhibitor, and from untreated controls, and also samples to which the substrate had been added.

Though distinctions could be drawn between the different samples after a few hours, according to the intensity of melanin development, they became more clearly marked at the end of twenty-four hours. There seemed to be no further increase in the amount of melanin deposited after about thirty hours.

It was concluded from a series of tests of the kind illustrated in Figure 55, that 0. 45 mgm. of phenylthiourea introduced into the silkworm, partially blocked the blood phenolase activity, whereas it was completely blocked by the introduction of 1.0 mgm. Moreover, the inference to be drawn from the results of these tests is that the inhibitor is inactivating the enzyme *in vivo*.

3. Effect of suppressing respiration upon cocoon construction

To elucidate further the effect of phenylthiourea upon the spinning behaviour of *Cynthia*, studies were next made on the depressant action of this compound on respiratory metabolism during the spinning period.

An important symptom of metamorphosis in *Cynthia*, as already described, is the striking fall in the rate of oxygen consumption which begins two and a half days before the onset of spinning. The injection of phenylthiourea into silkworms during this pre-spinning period would

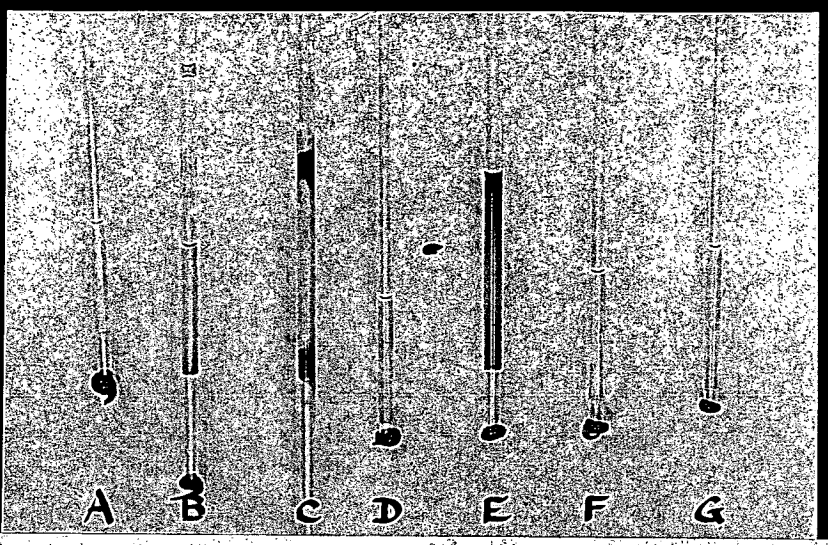

Figure 55. Photograph of samples of blood from *Cynthia* larvae illustrating the inhibitory effect of phenylthiourea on melanin formation. For details see text. A - solution of diphenol substrate; B - untreated blood; C - untreated blood incubated with the diphenol; D - blood from silkworm injected with 0.45mgm. phenylthiourea twenty-four hours previously; E - same as D, but incubated with the diphenol; F - blood from silkworm injected with 1mgm. phenylthiourea twenty-four hours previously; G - same as F, but incubated with the diphenol.

therefore be expected to depress further the already decreasing rate of oxygen consumption. It is also reasonable to assume that this fall in the respiration rate reflects the progress in the series of events characterising metamorphosis, which is triggered off by the "pupation" hormone (Williams, 1947). The insertion of phenylthiourea into the blood just prior to the spinning period was therefore considered unlikely to influence the normal progress of metamorphosis.

Accordingly, 5th instar Cynthia larvae were injected with various amounts of phenylthiourea, dissolved in insect Ringer solution (Ephrussi and Beadle, 1936). 0.5 cc. of solution was given in each case, containing amounts offrom 0.1 to 0.7 mgm. phenylthiourea, and was injected at the black-tip stage, about twenty-four hours before the onset of spinning. The larvae used in this experiment weighed between 2 and 3 grams at the time of injection.

The oxygen consumption of the treated larvae was measured over the period beginning approximately four days before the cessation of feeding, and ending at pupation or at the prior death of the animal. The rates of oxygen consumption were calculated on the basis of the pupal weight. In those cases in which a pupa was not formed, owing to the death of the animal, the "pupal weight" was estimated, using ratios of larval/pupal weights obtained from normal animals. The errors introduced by this procedure would show up as vertical shifts in the oxygen consumption graphs, falsifying the absolute values recorded, but would not affect the shapes of the curves.

Silkworms in control experiments spun normal cocoons after being injected with 0. 5 cc. Ringer solution.

Figures 56 and 57 show graphs of oxygen consumption of Cynthia larvae treated with various amounts of phenylthiourea at the black-

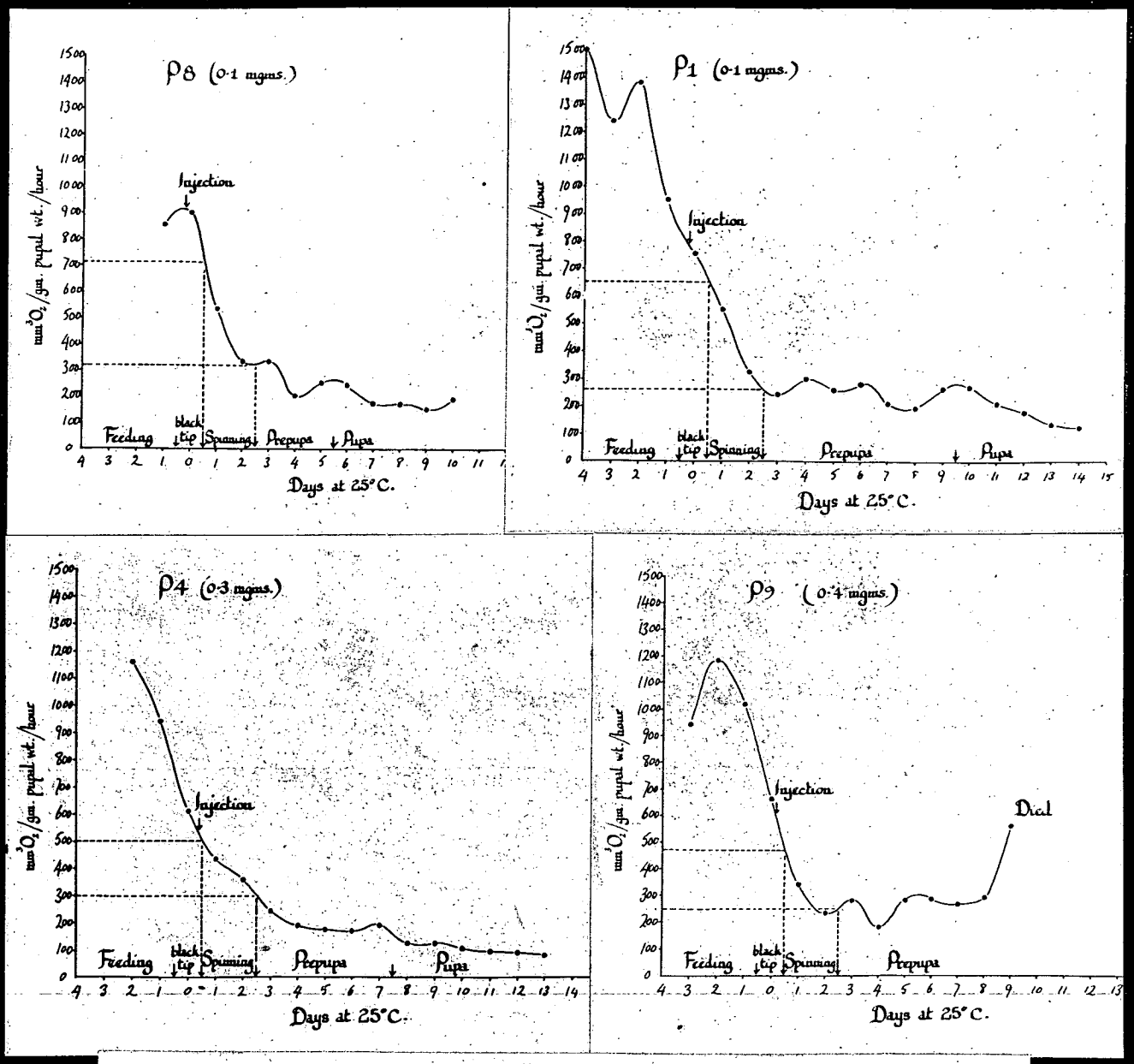

Figure 56. Graphs showing the changes in rate of oxygen consumption of Cynthia larvae, treated with various amounts of phenylthiourea at the black-tip stage, over the period of the onset of metamorphosis. The rates at the beginning and end of the spinning period are shown by the dotted lines. For details see text.

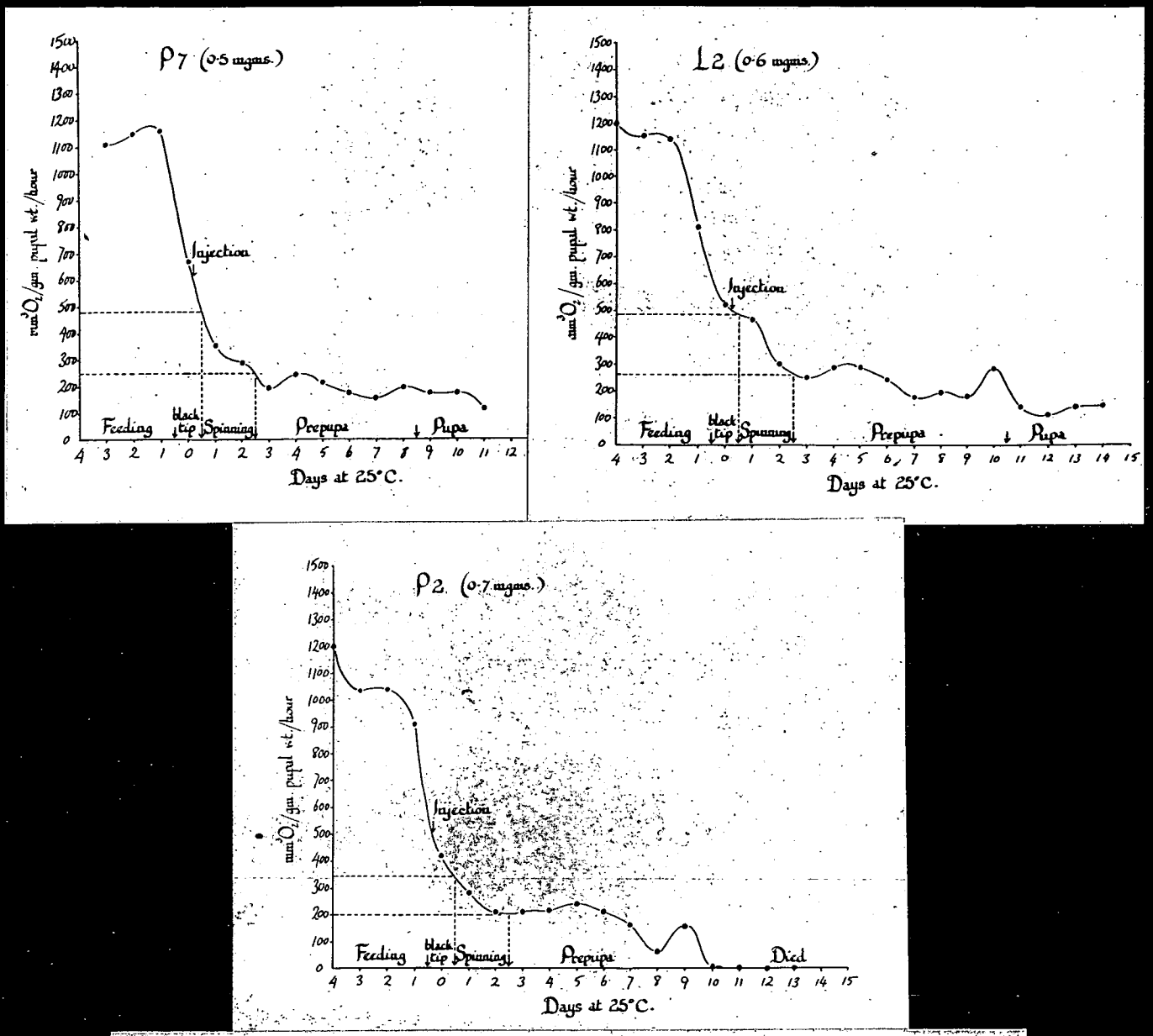

Figure 57. Graphs showing the changes in rate of oxygen consumption of Cynthia larvae, treated with various amounts of phenylthiourea at the black-tip stage, over the period of the onset of metamorphosis. The rates at the beginning and end of the spinning period are shown by the dotted lines. For details see text.

tip stage. It is clear that this treatment depresses the already decreasing rate of oxygen consumption, and that the depression is proportional to the amount of phenylthiourea injected. It is significant that the structures spun by the treated larvae show abnormalities related to the rate of oxygen consumption at the beginning of the spinning period, and that these abnormalities appear to be qualitative.

Table 2 shows the relationship between the amount of phenylthiourea injected, the rates of oxygen consumption at the beginning and end of spinning, and the types of cocoon spun. The latter are illustrated in Figure 58. The rates of oxygen consumption have been taken from the graphs, and although little weight can be placed on their exact accuracy, they adequately represent the overall trend.

The results may be considered in three groups. Firstly, the larvae treated with 0.1 mgm. spun closed cocoons superficially similar to the controls, by closer examination revealed that the structure consisted of only two layers of silk instead of the usual three (Figure 59, cf. Figure 1). The rates of oxygen consumption at the beginning of spinning in the two cases were 710 and 650 $mm^3 O_2$/ hour, and pupation was normal.

Secondly, the group treated with 0.3 to 0.5 mgm. The larva P9 was exceptional in spinning a "hammock" after receiving only 0.4 mgm., but, as it also died prematurely, it may have been particularly susceptible to the treatment. Larvae P4 and P7 showed much more sheet-spinning than the controls, and their cocoons were both flimsy structures, one with two layers of silk, and the other with only a single layer. Their rates of oxygen consumption at the

Animal number	Dose mgms.	rate of oxygen consumption $mm^3 O_2$/gm. pupal wt./hour			Cocoon type	Silk wt. mgms.	Pupal type
		start of spinning	end of spinning	range			
P6	~	740	250	490	Normal, 3-layered	83	Normal
P8	0·1	710	320	390	2-layered, closed	52	Normal
P1	0·1	650	260	390	2-layered, closed	114	Normal
P4	0·3	500	300	200	2-layered, open	89	Larval type pupa
P9	0·4	470	250	220	Hammock	21	Died as prepupa
P7	0·5	480	250	230	1-layered, open	55	Normal
L2	0·6	480	260	220	Hammock	69	Shrivelled
P2	0·7	340	200	140	Hammock	46	Died as prepupa

Table 2. Summary of the results of experiments in which phenylthiourea was injected into P. cynthia larvae just before spinning. The oxygen consumption figures have been taken from the graphs, figs. 46, 56 and 57. For details see text.

Figure 58. Photograph of the structures spun by Cynthia larvae after treatment with various amounts of phenylthiourea at the black-tip stage. The amounts of inhibitor injected into the larvae were: P6 - untreated control; P1 - 0.1mgm.; P8 - 0.1mgm.; P4 - 0.3mgm.; P9 - 0.4 mgm.; P7 - 0.5mgm.; L2 - 0.6mgm.; P2 - 0.7mgm.. For further details see text.

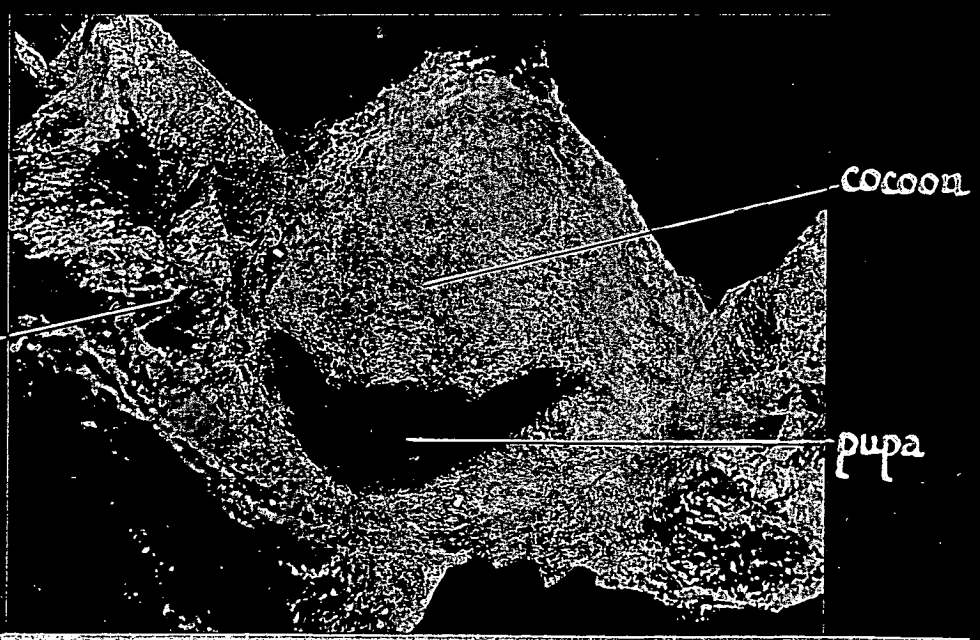

Figure 59. Cocoon spun by Cynthia larva treated with 0.3mgm. phenylthiourea at the black-tip stage, showing the capsule cut open to reveal the pupa inside. The capsule consisted of only two layers with a rough exterior surface, and was attached by the sheet silk to the inside of the glass vial where it was spun. (x1½ approx.) (cf. Figure 1)

the Discussion. Figure 60 shows one such case, which may be compared with the normal pupa illustrated in Figure 61.

E. Reducing metabolism by exposure to low temperatures and its effect on larval movement

Cooling is well-known as a method of reducing general metabolism, and so it was considered that the direct dependence of silkworm behaviour on general metabolism could be tested by this means.

First of all, therefore, feeding Cynthia larvae were placed in a chamber maintained at approximately 12°C., and it was observed that they were able to feed normally at this temperature. Next, larvae, which had just started to spin, were exposed to the same temperature. These larvae spun normal cocoons, and pupated successfully, although the duration of the spinning and prepupal periods was very much longer under these conditions, than at 25°C. It was therefore evident that the normal behaviour of the Cynthia larva is not affected by treatment at 12°C., apart from increasing the time-scale, and making the various movements slower.

The next experiment involved treating a number of feeding 4th instar Antheraea larvae for periods of up to two weeks in a refrigerator at 5°C. The larvae showed no spontaneous activity under these conditions, but, when removed from the refrigerator and placed at room temperature, spontaneous movements recommenced as the animals warmed up. These movements were closely observed in the hope of recognising any unusual behaviour.

After four days at 5°C., the larvae showed "searching" movements very soon after being brought out of the refrigerator, feeling the proffered food with their labial palps. They started to eat after

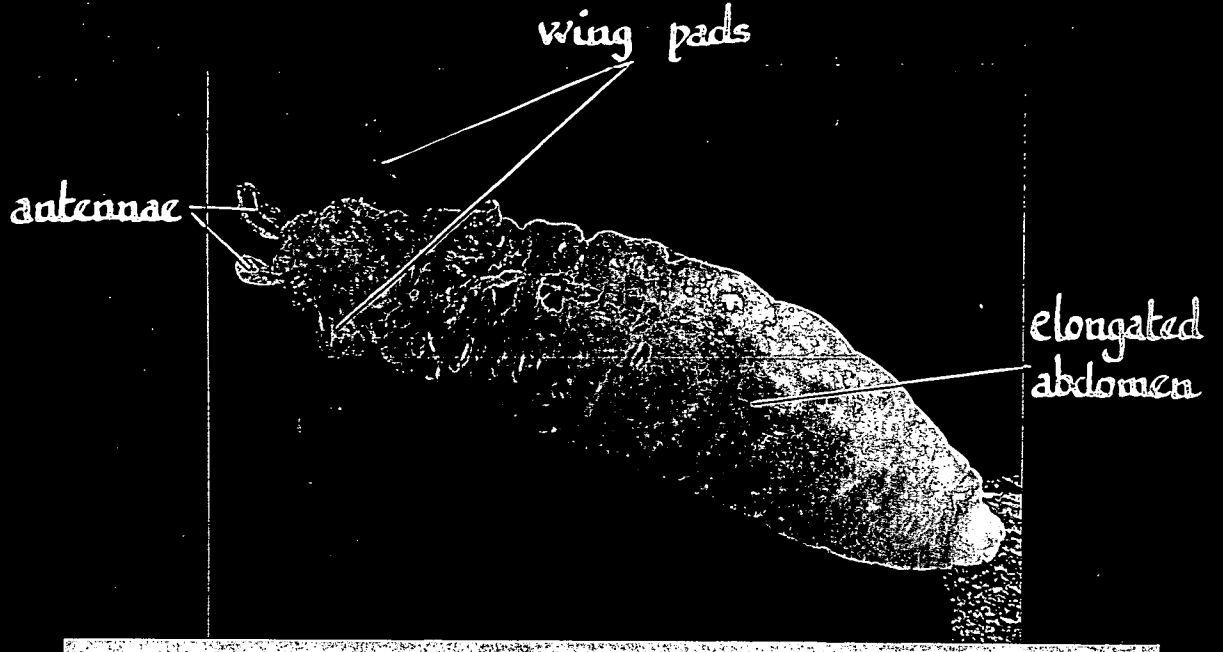

Figure 60. Cynthia, larval-type pupa formed after treatment with phenylthiourea. The cuticle has not yet hardened or darkened, but the elongated form, and the small antennae and wing-pads can be clearly seen. (x2 approx.) (cf. Figure 61)

Figure 61. Cynthia, normal pupa showing typical body shape, antennae and wing pads. (x3 approx.)

about fifteen minutes, and exhibited nothing unusual in their behaviour.

After seven days at 5°C., however, some larvae showed very few movements when returned to room temperature. They remained curled and on their sides, making only slight head movements when stimulated. Others either recovered quickly and began feeding, or showed a partial abdominal paralysis for a short time before recovering sufficiently to start feeding. A fortnight's treatment at 5°C. proved lethal to some of the larvae, whereas the others took rather longer to recover normal co-ordination, when removed from the refrigerator. Spinning movements with the spinneret protracted were never shown by these larvae.

It appears, therefore, that simple lowering of the metabolism by cooling, has no direct effect on silkworm behaviour, although it is evident that the nervous system can be seriously affected by exposures of a week or more to 5°C.

F. The brain in relation to respiratory systems

It is well known that the cortex of the human brain is very susceptible to oxygen lack, and that this fact explains the occurrence of "mountain sickness", and of the disorders in behaviour shown by airmen flying without additional oxygen at altitudes of over 10,000 feet. Without committing the crime of arguing directly from mammals to arthropods, it is possible that the brains of insects and arachnids may also be susceptible to anoxia. If this supposition is accepted for the moment, one can proceed to elaborate a theory whereby variations in the amount of oxygen available to the brain could directly cause changes in behaviour, possibly through the alteration of

nervous thresholds. With this theory, one could also explain how changes in behaviour are correlated to changes in the physiology of the animal, provided the latter affected the supply of oxygen to the brain. This latter point is the most difficult part of the theory to justify, and it may be argued that, in insects at any rate, the brain possesses its own particular tracheal supply and therefore could not be affected by metabolic changes in other parts of the body. This criticism is undoubtedly correct, and would prove fatal to the theory if it could be proved that the tracheal system is the only means whereby the brain obtains oxygen. In other words, if it could be shown that the blood plays an important part in the transport of oxygen to the tissues, and that metabolic changes in the body could alter the amount of oxygen carried, then the theory would at least be possible.

In the spiders, which have all the ganglia fused together in a mass lying in the centre of the prosoma, some families have well-developed tracheal systems, but in the orb-web spiders the system consists of four small tubes running forwards from the spiracle, and ending blindly within the abdomen. The blood circulation is so arranged that the heart receives mostly oxygenated blood direct from the lung-books, and pumps it forwards into the prosoma, where the aorta splits up into branches going to the ganglionic mass as well as to the other regions (Millot, 1949). It is clear, therefore, that all the organs lying in the prosoma, the ganglionic mass included, depend for their oxygen on that carried in the blood from the lung-books. As has already been suggested, therefore, obstruction of the blood flow by the vacuolated leucocytes which accumulate

before the moult, could be directly responsible for the change in behaviour which is observed at this time.

In the insects, however, the case for transportation of oxygen in the blood is not nearly so clear. It has been shown in some insects that the oxygen capacity of the blood is no higher than would be accountable for by physical solution, and that there is no evidence for the presence of respiratory pigments, except in a few specialised insects which possess haemoglobin. However, it would be rash to assume from this that the blood has been proved to have no important respiratory function, and indeed there are certain facts which suggest that this is a possibility.

Since the tracheal walls are permeable to gases (Wigglesworth, 1950), it is possible that gaseous exchange takes place between the blood and the tracheal air. It is also possible that cuticular respiration is important in soft-skinned larvae like the silkworm. Moreover, the brain lies just at the end of the aorta, and so receives blood directly from the heart. The evidence presented in this thesis concerning the action of phenylthiourea in depressing the rate of oxygen consumption in silkworms, however, suggests that a copper-containing enzyme is implicated in some way with respiration in this animal. At the present time it is not considered likely that blood phenolase plays a role in insect respiration, but this is by no means proved, and the correlation obtained between depression of oxygen consumption and inhibition of blood phenolase in Cynthia is cogent, although circumstantial, evidence for the contrary view.

Although it is beyond the scope of the present study to attempt to resolve this problem, it was considered that an investigation

might be made into the importance of the tracheal supply to the brain of the silkworm. Accordingly it was decided to determine the extent to which the tracheoles ramify within the brain itself, and also to estimate the dependence of the brain upon its tracheal supply.

1. Tracheation of the brain

Snodgrass (1935) reports that each half of the brain is supplied by a single tracheal branch, arising on each side from one of the large tracheal trunks running into the head from the first thoracic spiracle. Dissections were made to confirm this, and Figure 62 shows the brain and its tracheal branch in Cynthia.

This branch splits up within the brain, and its ramifications are shown in Figure 63. The preparation illustrated was made from the brain of a freshly killed Antheraea larva by mounting it in well oxygenated tap-water. Under these circumstances the tracheoles can be seen as black lines within the nervous tissue, and remain air-filled long enough for a photograph to be taken.

It appears from this study, that the tracheal supply to the brain is probably sufficient for its needs since it appears to ramify widely through the tissue.

2. Effects on behaviour of severing the tracheal supply to the brain

The tracheal supply to the brain was severed or blocked in a number of feeding 5th instar Cynthia larvae. This was accomplished either by filling the 1st thoracic spiracle on each side with a warm mixture of vaseline and wax, which solidified on cooling, or by making incisions just in front of these spiracles and severing the

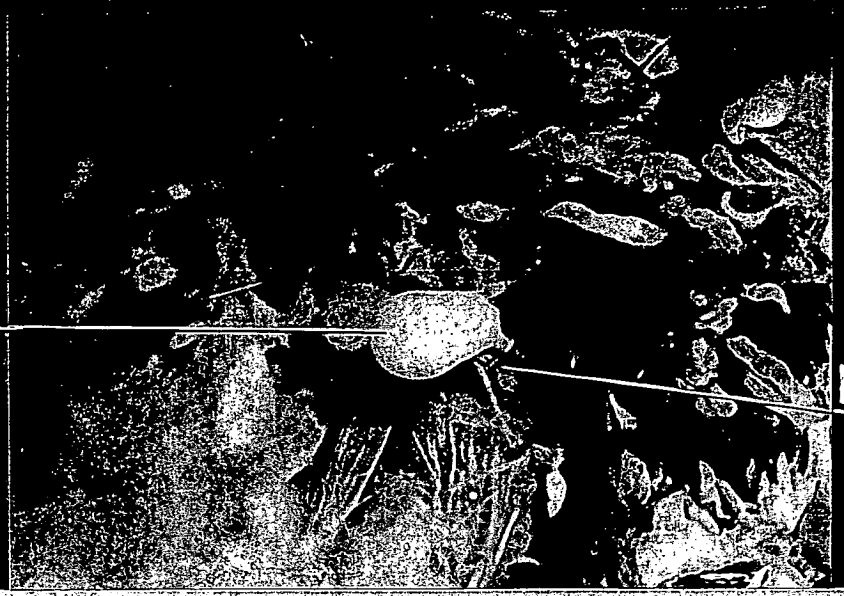

Figure 62. Cynthia, dissection of the larval head showing the tracheal branch running to the brain. (x25 approx.)

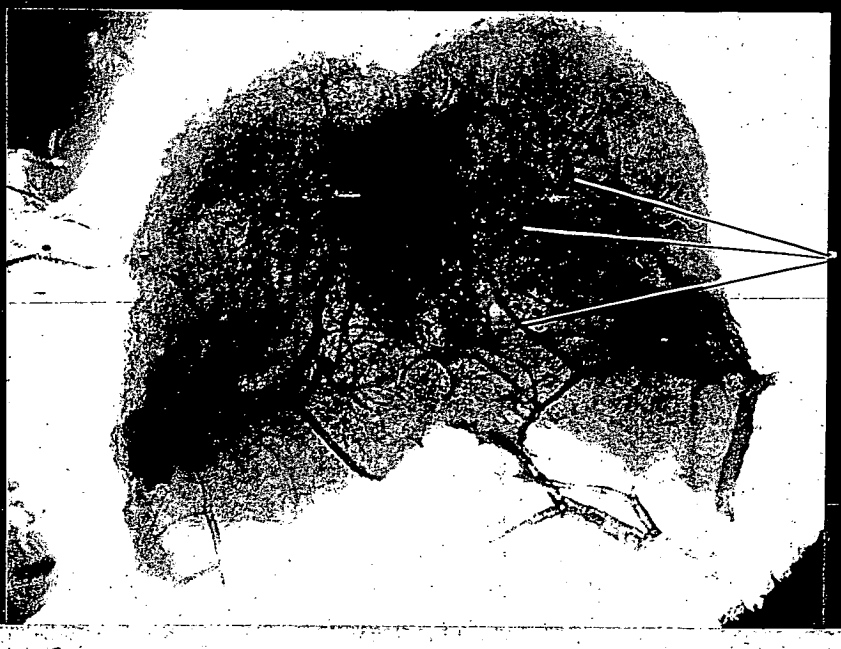

Figure 63. Antheraea, brain of freshly killed larva photographed by transmitted light, showing the internal tracheolar ramifications. (x100 approx.)

appropriate tracheae. The incisions were then closed with aluminium clips, and moistened with 96% alcohol.

All the larvae recovered rapidly from the effects of these treatments, and showed normal feeding behaviour for about a week afterwards. They then became moribund and died. It is interesting that these larvae could survive for this period after such a drastic operation, and the results suggest that the brain's metabolism does not depend solely on respiration via the tracheae. The final death of the larvae shows, however, that an intact tracheal supply is essential for the maintenance of life beyond this period. Nevertheless, it may be mentioned that the time at which these larvae died, corresponded to the approximate time at which they would have entered metamorphosis had they been left untreated. It is therefore possible that the physiological changes of metamorphosis cause the brain to rely completely on tracheal respiration at this time, by effectively removing the other (hypothetical) means for accomplishing gaseous exchange.

DISCUSSION

1. Silk spinning

The results of the experiments on spiders designed to throw light on the relationship between the speed of production of the dragline silk, and the tension required for its extraction, reveal two interesting features. Firstly, each of the graphs, plotted from readings obtained in these experiments (Figure 38), show a linear portion, the slope of which, however, varies widely between the different graphs. Secondly, each graph, with only one exception, shows a "critical speed" at which the linear relationship between the silk tension and the speed of extraction breaks down.

The slope of the graph is a measure of the frictional resistance in the duct, and, since the variation in slope bears no relationship to the weights of the individual spiders, it would appear that the frictional resistance can vary independently of the body size, and hence of the size of the duct. The problem now arises of how these variations have been produced in the experiment, and it is necessary at this point to consider the reasons which led up to the choice of ether as an anaesthetic.

Preliminary experiments using carbon dioxide were abandoned because it seemed to make the operation of extracting the silk very difficult. When the silk was picked up on a needle, the production of the dragline ceased about half a minute after the spider had been fully anaesthetised, whereas the other types of silk could be picked up and pulled out for longer periods. On trying ether as the

anaesthetic, however, it proved possible to pull the dragline out continuously, after it had been picked up. It seemed possible, therefore, that anaesthetisation with ether resulted in incomplete relaxation of the muscles controlling the opening of the control valve, whereas carbon dioxide brought about the complete relaxation of these muscles. The results of using ether would therefore be to prevent the complete closure of the valve, thus allowing the silk to pass; but with carbon dioxide the valve would close, and the silk would be held back. It can be seen, however, that the amount of relaxation achieved under ether would vary depending on the previous activity of the spider, its physiological state, and so on, and it is considered that the variations in slope shown by the graphs can be explained in this manner.

In view of the variations of duct friction which have been discussed above, it is all the more striking that the "critical speed" remains at so constant a value of 30 cms/second. This demonstrates that it is an effect largely independent of the state of muscular relaxation. The experimental results suggest that the duct may collapse and grip the silk at this speed, possibly due to the reduction of pressure associated with a "venturi effect" at the valve lips. Alternatively, the flow of silk may suddenly become turbulent, with a consequent great increase in the frictional resistance. As this effect would automatically stop the production of the dragline when a spider drops at a speed higher than about 30 cms/second, it is considered that the limited fall observable when the spider drops from its web could be attributed to the above phenomenon.

Whereas, in the orb-web spider, the control valves in the ducts of the dragline glands seem to be opened by the attached muscles, and closed by the elasticity of the duct itself, it is interesting to find that the silk press of the silkworm is operated in a similar manner (Snodgrass, 1935). While attempting to pull silk forcibly from a spinning silkworm, it was found that only a limited amount could be extracted before the flow of silk was held up. Continued pulling resulted in the silk breaking off at the spinneret. Neither was it possible to pull the silk out when the larva was relaxed under an anaesthetic.

It seems then that the flow of silk is halted when the muscles of the press are relaxed, either naturally, or artificially by the action of an anaesthetic. It has previously been suggested that the function of the press is to squeeze the two strands of silk together so as to form a double thread (Ho et al., 1944: Imms, 1949), but this is unlikely, since closure of the press results in stopping the flow of silk altogether. Moreover, when the two sticky threads pass through the press and spinneret, they would form a double thread without any squeezing action being necessary.

The silk press therefore seems to act as a control valve which regulates the flow of silk, as does the control valve of the orb-web spider.

2. Depressant action of phenylthiourea on respiration

Since cytochrome oxidase is immune to the action of various urea compounds (Grant and Krantz, 1942) and of phenylthiourea (Dubois and Erway, 1946), it is of considerable interest that the

latter compound depressed respiration in Cynthia larvae, the depression being approximately proportional to its concentration in the blood.

Phenylthiourea has been used for some time to prevent the formation of toxic phenolic substances after surgical operation on insects (Williams, 1952), since, as a copper-catalyst blocking agent, it inhibits the action of blood phenolase.

Since the presence of 1 mgm. of phenylthiourea in the blood of Cynthia larvae inhibits the phenolase activity, and at the same time completely blocks respiration in the intact larva, it would seem that phenolase might have a role in insect respiration as suggested by Heller (1947). It has been shown, however, that the silkworm possesses an intact and fully functional cytochrome-cytochrome oxidase system (Schneiderman and Williams, 1954; Shappirio and Williams, 1957a, b). Previously, the cyanide and carbon monoxide insensitivity of the metabolism of diapausing pupae, suggested the possibility that tyrosinase might be serving as a terminal oxidase in respiration, instead of cytochrome oxidase. Sussman (1949, 1952) however, showed that this was not the case.

Morton (1958) has recently drawn attention to the wide distribution of quinone compounds in tissues, and Slater (1958) has suggested how these compounds may be involved in oxidative phosphorylation. The evidence up till now is in favour of this, and it is therefore tempting to suggest that, if the quinones in question are derived from quinol compounds only in the presence of an active phenolase-type enzyme, then blocking this catalyst would be expected to have an inhibitory effect on oxidative phosphorylation.

In spiders, the tracheal system is relatively poorly developed, and, although no direct evidence is available on this point, the internal anatomy suggests that oxygen carried in physical solution in the blood, may not be sufficient to support all the metabolic processes of the animal. It would therefore be interesting to learn whether the blood phenolase of spiders has a respiratory role.

The well-developed insect tracheal system would seem to preclude the necessity for the blood to play an active part in the transport of oxygen in these animals. It has, however, been suggested that the blood must "play the part of intermediary between the lumen of the tracheae and the cytoplasm of the cells" (Wigglesworth, 1950), and the possibility that blood phenolase may serve as a terminal oxidase at these sites cannot therefore be ruled out.

3. The control of spinning behaviour

A reduction in the rate of oxygen consumption is an important sympton both of the moult and of metamorphosis in the silkworm. This fall has been recorded for Cecropia at metamorphosis by Schneiderman and Williams (1953), and these authors state that the decrease in metabolism begins after the cocoon has been spun. The results presented in this study show, however, that spinning behaviour is initiated after the rate of oxygen consumption has already fallen to a relatively low level, and thus, in Cynthia, the reduction of general metabolism is associated with the onset of spinning. Epidermal and cuticular changes which form an important part of moulting probably account for the cessation of spinning and the onset of the quiescent prepupal period which follows.

The larva stops feeding before spinning occurs, and, in the

case of metamorphosis, the gut is emptied of its contents, and the food last eaten is passed out as green and undigested leaf. At the larval moult the gut is not emptied completely, although digestion ceases, and it has been shown by dissection that green, undigested fragments of leaf remain in the gut. Thus the fall in rate of oxygen consumption is associated with a suspension of gut function.

It may be that all the larval tissues show a reduction in metabolism at this time, and that the measurement of oxygen consumption reveals a general condition, such as the partial breakdown of the cytochrome system(Sanborn and Williams, 1950: Shappirio and Williams, 1957a, b). On the other hand, it might be argued that, as the gut is an organ of major importance in the larva, the level of oxygen consumption reflects mainly the level of gut metabolism. It has been shown that the effect of withholding food from larvae in the feeding stage resulted in a very rapid decline in the rate of oxygen consumption in Antheraea. Indeed, the rapidity and extent of the fall is more than adequate to account for the declines associated both with the larval moult and with metamorphosis.

If the gut metabolism controls the decline in oxygen consumption, then it must also account for the rise which occurs when moulting has been completed. This rise begins before the larva starts feeding again, and must therefore be due to the digestion of the food which remains in the gut during the moult. The larva has been observed to pass faeces during this period, which supports the view that, after the moult, the gut starts functioning before feeding is resumed.

It appears then as if the overall oxygen consumption in the

silkworm reflects the gut metabolism, which in turn depends on the quantity of food present, and that this overshadows the other metabolic processes in the larva.

Dethier and Bodenstein (1958) have shown that chronic hyperphagia results from section of the recurrent nerve anterior to the hypocerebral ganglion in the blow-fly, Phormia. By a series of elegant operations on the gut and its innervation, they have also demonstrated the system of proprioceptive inputs to the brain from the various regions of the gut, which control ingestion and the movement of food. It is likely that in the silkworm also, distension of the crop or foregut controls ingestion during the feeding stage, and it would be interesting to record the activity of the recurrent nerve during metamorphosis, in order to investigate whether the change in input to the brain, which must occur when feeding stops, could mediate the spinning behaviour. Future work is planned to include experiments dealing with this problem.

The structures spun by Cynthia larvae after treatment with various amounts of phenylthiourea just before spinning, fall into three groups. These correspond to different levels of oxygen consumption at the beginning of spinning, the least normal being constructed at the lowest level of oxygen consumption. If the metabolism of the brain is altered in a manner which parallels the measured oxygen uptake, then it could be argued that a hierarchical system of organisation exists in the brain (Tinbergen, 1951: Thorpe, 1956), the most complex behaviour occurring under conditions of maximum brain metabolism. As metabolism is reduced, the pattern of nervous activity would alter, thereby producing progressively simpler and more stereotyped behaviour.

This theory might be applicable both within spinning behaviour as such, where the most complex spinning movements occur at the higher metabolic levels during the spinning period, and also to the difference between feeding and spinning, since the latter activity seems to be less complex than the former, and invariably occurs at times of low metabolism. That behaviour might be controlled by metabolism has already been suggested some time ago in relation to changing photosensitivity in Mayfly nymph (Allee and Stein, 1918).

Moulting is initiated by the prothoracic gland hormone when the titer reaches a certain critical level (Williams, 1947). The tissues react to this in a manner controlled by their competence, physiological age, and also by the titer of juvenile hormone. In the presence of sufficient juvenile hormone, the cells will differentiate into larval type: if the juvenile hormone titer is too low, however, the cells will differentiate to form pupal tissues (Williams, 1952: Bodenstein, 1953: Wigglesworth, 1954). Intermediate conditions of hormone titer lead to the production of mixed or intermediate types of tissue (Piepho, 1950). It has also been shown that, when insufficient prothoracic gland hormone is present, only part of the epidermis will transform to pupal cuticle (Kühn and Piepho, 1938). The fact that larval-type pupae were sometimes produced after treatment with phenylthiourea, therefore, indicates that this substance in some ways acts similarly to the juvenile hormone.

Fukuda (1951) states that larval Bombyx mori, ligatured just behind the head about three days after entering the 4th instar, "mature" precociously after 96 hours, and become precocious pupae after 168 hours. If the rate of maturation is the same in Cynthia, then under normal conditions the corpora allata stop producing

hormone about two days before spinning, which coincides with the beginning of the fall in metabolism that probably signals the decline in gut function.

In making tissue culture assays of the prothoracic gland hormone, Schmidt and Williams (1953) added sufficient phenylthiourea to their culture medium to block phenolase activity. This technique indicates that the prothoracic gland hormone is unaffected by phenylthiourea, and it would therefore seem that the injection of this compound on the day before spinning would have little effect on the hormone balance in the larva. It must therefore be assumed that the formation of larval-type pupae resulting from this operation is due to an effect on the developing cells, and that the abnormal structures spun possibly result from the direct effect of phenylthiourea on the brain.

It may be significant that, whereas 0.7 mgm. phenylthiourea inserted as crystals into feeding Cynthia larvae led only to a temporary lowering of metabolism, the same amount injected just prior to spinning led to complete collapse and death in the prepupal stage. The larvae so treated did not contract as is typical of this stage in metamorphosis, and remained green for about two months after death, indicating that further development, together with all phenolase activity, has been stopped.

These results suggest that phenylthiourea has a greater effect on rapidly growing tissue than on resting tissue, a conclusion which is supported by the experiments of Jenkins (1959) on regeneration in planarians.

Although the relationship between metabolism, hormones, phenolase and phenylthiourea offers much opportunity for interesting

speculation, this lies beyond the scope of the present work.

The experiments of Van der Kloot and Williams (1953b) on the spinning behaviour of glandectomised Cecropia larvae, led them to conclude that "the presence of the silk glands provides the stimulus for the initiation of the spinning movements". They interpreted the figure-of-eight movements shown, as visual orientation movements similar to those of larvae removed from the food plant (Dethier, 1943).

Close observation of glandectomised Cynthia revealed, however, that the spinneret is held forward, and is wiped over the substrate during the figure-of-eight movements. Continuous protraction of the spinneret characterises the spinning of normal larvae, and is only occasionally seen during the feeding stage. The searching movements of the feeding larva, although superficially similar to the figure-of-eight spinning movemants, are carried out using the labial palps to investigate the substrate. It therefore seems reasonable to assume that the figure-of-eight movements shown by glandectomised larvae are indeed spinning movements, as proposed by Umeya (1926).

Glandectomy, however, alters cocoon-spinning behaviour to a great extent; for instance, the movements used in spinning the outer capsule are absent. Van der Kloot and Williams (1953b) have shown that Cecropia larvae performed almost normal spinning spinning movements after their spinnerets had been blocked at the beginning of spinning. The difference in behaviour shown by glandectomised larvae and those with their spinnerets blocked have led them to deduce that the initiation of cocoon-spinning depends,

at least in part, on sensory input arising from the spinning apparatus itself.

The lesser activity of larvae glandectomised early in the last instar as compared with that of those operated on at the beginning of spinning, may be due to a toxic effect resulting from the accumulation of aminoacids in the blood of the former (Akao, 1943). The latter's blood, however, must also show an accumulation of aminoacids, though to a lesser extent, because, under normal conditions, the glands synthesise about 20 to 25% of the total silk during the spinning period itself (Fukuda, 1951: Van der Kloot,and Williams, 1953b).

The silk glands could also influence the brain, and through it the spinning behaviour, by proprioceptive input dependent on the turgidity of the gland. It has been shown, however, that Cynthia larvae, with all except the tips of the secretory part of the glands removed, perform essentially normal spinning movements. This operation effectively rules out gland turgidity, together with any associated sensory input to the brain, as a necessary requirement for normal spinning behaviour.

As there is little evidence that the silk glands function as endocrine organs (Umeya, 1926), these facts lead us to the conclusion, that the only influence glandectomy has on spinning behaviour is through the toxic effects of aminoacid accumulation, and that even a small portion of the gland left in the body is sufficient to maintain approximately normal conditions in the blood.

A comparison of the oxygen consumption between normal and glandectomised Cynthia larvae shows that the removal of the silk

glands causes an overall lowering of the rate during the onset of metamorphosis. Since the silk glands are metabolising very actively at this time, it is possible that the effect shown is due simply to a reduction in the amount of metabolising tissue in the larvae, coupled with the breaking of the associated tracheal branches, and not to any influence the operation may have had on the rest of the body.

It has been demonstrated that spinning occurs only at relatively low and falling rates of oxygen consumption; that glandectomy, which reduces oxygen consumption at the time of spinning, causes a marked alteration in the behaviour; and that the structures spun after phenylthiourea injection are related to the oxygen consumption at the beginning of spinning. It would therefore be tempting to suggest that spinning behaviour in the silkworm is directly dependent on general respiratory metabolism.

Cynthia larvae can survive and behave normally for up to a week after the tracheal supply to the brain has been cut. This suggests that the brain is receiving oxygen from the blood, in order to maintain its metabolism, although little is known of the capacity of insect nervous tissue to contract oxygen debts or to maintain anaerobic respiration. The tracheal system could act as a "ramifying lung", and gaseous exchange could be accomplished through the tracheal walls, since these are known to be permeable to gases (Wigglesworth, 1950). If the blood could be shown to have a role in the transport of oxygen, then it would be possible for brain activity to be linked directly to metabolism, in this way achieving precise co-ordination between physiology and behaviour.

Unfortunately the available evidence does not favour this

hypothesis. The ultimate death of the larva proves that the direct tracheal oxygen supply to the brain is essential, while the extensive ramifications of the tracheoles within the nervous tissue also indicate the importance of such a supply. Furthermore, it has been shown that an air-filled trachea, even if free communication with the outside has been interrupted, can still provide an efficient diffusion path for the respiratory gases (Buck and Keistner, 1956). The latter may well explain the limited survival of the larvae with the tracheae to the brain cut, death occurring after the tracheae had filled with body fluid.

If brain activity is indeed directly dependent on general respiratory metabolism, it should be possible to cause feeding silkworm larvae to make spinning movements by artificially lowering their metabolism. When feeding Antheraea larvae were placed at 5°C. for periods ranging up to two weeks, and were then allowed to recover at room temperature, no typical spinning movements were observed. The larvae showed searching movements, and fed when they had recovered after about a quarter of an hour. Some of the larvae, however, showed a marked debility when returned to room temperature after a week at 5°C., their abdomens were paralysed and, when touched, they responded only by making slight head movements.

It is also of interest that the nervous co-ordination of larvae starved for about nine hours at 25°C. was seriously upset. Their rate of oxygen consumption was very low, and they showed spasmodic twitchings, a semi-paralysis of the abdomen, and badly co-ordinated movements. Indeed, one larva showed no movements

other than moving its head from side to side with spinneret protracted. The other larvae, however, showed only searching movements, feeling the substrate with their labial palps, and started to feed when presented with a leaf.

4. The relation of metabolic rate to behaviour

It will be apparent already that spinning behaviour in the silkworm is linked with the changes in metabolism which occur at the moult and at the onset of metamorphosis. It is also clear that spinning is invariably coupled with a low and falling metabolic rate, while feeding is coupled with a high level of metabolism. Moreover, it appears as if the cocoon-spinning movements themselves are arranged with respect to the metabolic rate, so that the most complex movements occur at the beginning of the spinning period when the oxygen consumption is highest.

Since simple lowering of the general body metabolism of a feeding silkworm, for instance by reducing the body temperature, does not induce the performance of spinning movements, it can be concluded that the relationship between the level of metabolism and the spinning behaviour is not a direct one. Indeed, it would be too much to hope that the workings of such a delicate organ as the brain could be affected by such a crude experiment, so as to produce a limited and precise alteration in its activity.

Nevertheless, evidence has been presented that the respiration of the brain may depend partly on oxygen carried in the blood, and so might be influenced by somatic changes which affect this supply of oxygen. It is recognised, of course, that this

hypothesis rests solely on circumstantial evidence, but it is considered that it would merit more serious attention were it proved, at some later date, that the blood of insects plays an active part in the respiration of these animals.

Leaving aside this question, therefore, attention must next be focussed on the possibility that the relation between spinning behaviour and the general metabolic level might be through the medium of a third factor, on which they both depended. It must be remembered that the measured oxygen consumption merely gives an indication of the overall metabolism, and that changes in the rate may be attributable to a reduction in the metabolism of a particular organ, as well as to a general metabolic depression.

It has previously been considered that the striking fall in metabolism which occurs at the beginning of metamorphosis is due to the breakdown of the cytochrome system throughout most of the larval tissues (Sanborn and Williams, 1950: Shappirio and Williams, 1957a, b). Although no direct evidence is available, however, it is unlikely that this is the cause of the fall in respiration which occurs before the larval moult Moreover, the results of the present work suggest strongly that changes in gut metabolism could overshadow other metabolic effects, and account for the observed variations in rate of oxygen consumption, both at the moult and at the onset of metamorphosis. If this evidence is accepted, therefore, it would be logical to examine whether the brain could be influenced directly by changes in the metabolism of the gut, so as to produce the observed behavioural changes.

Undoubtedly the metabolism of the silkworm is under the ultimate control of the hormones, and it might be pointed out that, although the hormone balance which induces moulting is different from that at metamorphosis, so are the structures spun different at these two times. It is therefore possible that spinning is initiated by, say, the prothoracic gland hormone acting directly on the brain, but that the structure spun depends on the presence or absence of juvenile hormone. No evidence given in this thesis actually disproves the above hypothesis, and so it must remain as a possibility until further experiments either verify or disprove it. Whether the influencing of the brain by a hormone, which is itself secreted under influence from the brain, is considered a likely arrangement, must be left to individual taste.

To complete this review of the factors which might bring about the observed linkage between general body metabolism and behaviour, it must be mentioned that the silk glands are not considered to influence the brain directly by their presence or absence. The experimental evidence indicates that spinning behaviour is affected neither by a humeral secretion from the glands, nor by proprioceptive input from the spinning apparatus, but that the observed effects of glandectomy are due to "aminoacidaemie" (Akao, 1943).

Although the connections between general metabolism and behaviour still remain undecided in the silkworm, it is important to emphasise that the metabolism of the brain may itself play a critical part in determining the behaviour of insects, and also,

possibly, of spiders. The ease with which these animals change from one behaviour pattern to another suggests that all these patterns exist at the one time in the brain, and are switched in and out as required. The switching mechanism involved could very likely be changes in brain metabolism; more complex behaviour occurring when metabolism is high, and simpler, more stereotyped patterns of activity appearing as the metabolism is lowered.

This thesis has mainly been concerned with evaluating the possibility that metabolism plays an important part in controlling spinning behaviour. It would be rash, however, to assume that measurement of the overall oxygen consumption gives a full picture of the detailed metabolic processes which go on inside a living body, and it must therefore be recognised that only the fringe of this subject has been touched in the present work.

SUMMARY

1; A control valve in the duct of the major ampullaceal gland has been described in Araneus and Zygiella. It consists of two lips which point in the direction of silk flow and are attached by tonofibrillae to a set of muscles.

2. The valve appears to close under its own elasticity, although it is opened by direct muscular action. It is therefore functionally analogous to the "silk press" in the silkworm.

3. When the dragline silk is pulled out of anaesthetised spiders it is found that there is a "critical speed" of about 30 cm/second, at which the tension/speed relationship becomes non-linear. Frequently the silk "siezes up" and breaks at this speed.

4. Spinning of the cocoon by Cynthia and of the moulting pad by Antheraea occur at relatively low levels of oxygen consumption. The precipitous falls in the rate of oxygen consumption symptomatic of both moulting and metamorphosis, begin near the end of the feeding stage in each case.

5. Whereas at the onset of metamorphosis the gut is completely emptied, at the larval moult some undigested food is retained in the gut.

6. Starvation causes a spectacular fall in the rate of oxygen consumption of Antheraea, and can bring about a temporary semi-paralysis.

7. Glandectomy brings about an overall lowering of oxygen consumption in Cynthia at the onset of metamorphosis, and induces the larvae to show only figure-of-eight movements during the "spinning period". Since the spinneret is protracted during

their performance, these movements have been classed as true spinning movements.

8. It appears that spinning behaviour is not directly affected by sensory or proprioceptive input from the spinning apparatus.

9. It is suggested that sensory input to the brain from the gut may control spinning behaviour in the silkworm.

10. Phenylthiourea has a pronounced depressant action on the rate of oxygen consumption of Cynthia larvae, the depression being proportional to the concentration of this compound in the blood.

11. The decrease in respiration brought about by phenylthiourea coincides with the inhibition of blood phenolase activity.

12. When the rate of oxygen consumption is depressed to various levels at the beginning of spinning, a series of qualitatively different cocoons are produced.

13. Being kept at 5°C. for two weeks proved lethal to some Antheraea larvae, and produced temporary semi-paralysis in others.

14. Cynthia larvae continue to feed normally for one week after the tracheae to the brain have been blocked or severed.

15. It is suggested that changes in behaviour of spiders and silkworms may be brought about by variations in brain metabolism, which may depend on the general level of body metabolism.

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PUBLICATION OF RESULTS

A paper, entitled "THE EFFECT OF VARYING RESPIRATORY METABOLISM ON COCOON SPINNING BY THE CYNTHIA SILKWORM" was read at the Swansea conference of the Society for Experimental Biology, April 1959.

A second paper written in conjunction with Dr.B.M.Jones, entitled "STUDIES ON THE ACTION OF PHENYLTHIOUREA ON THE RESPIRATORY METABOLISM AND SPINNING BEHAVIOUR OF THE CYNTHIA SILKWORM", has been accepted for publication by the editors of the Biological Bulletin, Wood's Hole, Massachusetts, U.S.A.

Both these papers include an account of the experimental results given in the section of this thesis entitled "Respiratory metabolism and spinning behaviour in silkworms".